MODELOS DE EVOLUCIÓN MOLECULAR EN EL ESPACIO FENOTÍPICO DE FISHER:

TEORÍAS GENERALES DE LA EVOLUCIÓN MOLECULAR, EL ROL CREATIVO DE LA SELECCIÓN NATURAL Y EL ROL DE LA PLEIOTROPIA GÉNICA EN LA TASA DE EVOLUCIÓN MOLECULAR

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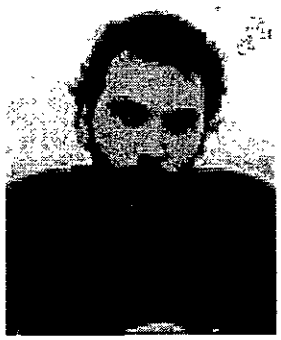

Pablo nació en Roma, Italia, en el año 1977, retornando con su familia a Chile en 1980. A través de su infancia y adolescencia mostró un interés permanente por las ciencias y la filosofía. Aunque de carácter más humanista durante su etapa escolar, supo de los problemas abordados por la física (la mecánica cuántica, la teoría de la relatividad) y atraído por ellos ingresó en 1996 a la Facultad de Ciencias de la Universidad de Chile donde se licenció en Física en el año 2000. Durante sus estudios de física comenzó a asistir paralelamente a cursos de biología, disciplina que cautivó su pasión científica y que le permitiría aplicar algunas herramientas aprendidas de la física. Se licenció en Biología en la misma Facultad de Ciencias en el año 2002. Participó por cuatro años en las discusiones sobre biología evolutiva y características sistémicas de los seres vivos organizadas semanalmente por el Grupo Decenio en el laboratorio fundado por Humberto Maturana. También participó en investigaciones en Neurobiología aplicando modelos matemáticos a fenómenos oscilatorios del sistema nervioso. Durante este tiempo estudió paralelamente en la Universidad Alberto Hurtado donde se licenció en Filosofía en el año 2005 y posteriormente obtuvo el grado de Magister en Estudios Filosóficos el año 2006, centrando sus investigaciones en la relación entre la Filosofía de la Biología y la Bioética. En esta universidad comenzó a dictar cursos sobre la relación entre las ciencias y la filosofía, consumando su permanente interés por los fundamentos de las teorías científicas, el cual se manifestaba en su constante asistencia a cursos de filosofía mientras estudiaba en la Facultad de Ciencias de la Universidad de Chile. En el año 2005 ingresa al programa de Doctorado en Ciencias con mención en Ecología y Biología Evolutiva en esta misma Facultad, lugar donde también dictó cursos de Filosofía de la Ciencia.

Dedicada a mis padres

TABLA DE CONTENIDOS

RESUMEN..... 1

ABSTRACT..... 4

INTRODUCCIÓN: EL MODELO GEOMÉTRICO DE FISHER..... 7

CAPÍTULO 1: MODELOS GENERALES DE LA EVOLUCIÓN MOLECULAR EN EL ESPACIO GEOMÉTRICO DE FISHER

Sección 1: The nearly-neutral and selection theories of molecular evolution under the Fisher's geometrical framework: substitution rate, population size and complexity.... 21

CAPÍTULO 2: EL ROL CREATIVO DE LA SELECCIÓN NATURAL

Sección 1: Tests of adaptation: what do and do not prove..... 61

Sección 2: The creativity of natural selection and the accumulation of advantageous substitutions by chance..... 161

CAPÍTULO 3: EL ROL DE LA PLEIOTROPÍA EN LA EVOLUCIÓN MOLECULAR

Sección 1: The rate of molecular evolution and the phenotypic complexity of mutational effects..... 199

CONCLUSIONES FINALES..... 232

APÉNDICE A: Generation of random vectors in an n-dimensional sphere..... 237

APÉNDICE B: Probabilistic causation and the explanatory role of natural selection..... 248

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RESUMEN

Los modelos de evolución a nivel molecular suelen no contemplar los efectos que las mutaciones tienen sobre el fenotipo, exceptuando el efecto neto sobre la adecuación biológica. El modelo geométrico de Fisher (FGM, en su abreviatura en inglés) es una excepción al respecto y en los últimos quince años se ha incrementado notablemente su desarrollo y el número de publicaciones que lo utilizan para abordar variados problemas en la biología evolutiva (véase Introducción). En esta tesis se utiliza el FGM para abordar tres problemas que se entroncan en cuestiones clásicas de la teoría evolutiva y que siguen abiertos hasta la fecha.

El primer problema aborda los objetivos de las teorías generales de la evolución molecular, cuyas preguntas principales son: cuál es la fuerza evolutiva predominante en la evolución (mutación, selección, deriva), qué proporción de tipos de mutaciones son sustituidas (neutrales, casi-neutrales, ventajosas, deletéreas), y cuál es la relación entre la tasa de evolución molecular y el tamaño de las poblaciones (independiente, creciente o decreciente). En el Capítulo 1 se introduce un modelo de evolución molecular en el FGM para caracterizar tanto un proceso evolutivo casi-neutral balanceado en un ambiente estable, como un proceso selectivo con variabilidad ambiental. Los resultados mostraron que el modelo balanceado predice una tasa evolutiva decreciente con el tamaño poblacional, al igual que otros modelos casi-neutrales, pero descarta que las mutaciones compensatorias puedan explicar la alta tasa de sustituciones ventajosas encontradas actualmente en los estudios de ADN de diferentes especies, como se ha supuesto previamente. El modelo selectivo, por su parte, predice una independencia de la tasa de sustituciones efectivamente ventajosas con el tamaño poblacional, lo cual es consistente con estudios empíricos

recientes en *Drosophila* que no se han sabido interpretar. En ambos modelos, se encontró que existe una relación estrecha entre parámetros clave de la evolución molecular, debido a factores que pueden interpretarse en términos biológicos y que apoyan un replanteamiento de la arbitrariedad con que suelen determinarse los parámetros en otros modelos de evolución molecular. Para llevar a cabo las simulaciones fue necesario perfeccionar previos métodos de generación de vectores aleatorios en un espacio multidimensional (Apéndice A).

El segundo problema refiere al rol creativo de la selección natural, un debate que comienza tras la publicación de Darwin del *Origen de las Especies* en 1859 y que continúa hasta hoy. Se ha cuestionado si la selección natural puede explicar el origen de los rasgos de los organismos en la naturaleza, pero el debate, tanto a favor como en contra, se ha dado principalmente en términos metafóricos y con poco sustento en pruebas empíricas. Por ello se hizo una revisión de los métodos existentes a la fecha para probar adaptación en poblaciones naturales (Capítulo 2, Sección 1), donde se encontró que no existe en la actualidad un método de prueba cuyo objetivo sea probar el rol de la selección natural en la formación de rasgos, aún cuando recientemente ciertas aproximaciones se han podido combinar para apuntar en esa dirección. En efecto, los métodos se han focalizado en evaluar el valor adaptativo de los rasgos, es decir, en la mantención o propagación (y por lo tanto, también en la eliminación) de adaptaciones. En la Sección 2 del Capítulo 2 se introduce un modelo donde se intenta estudiar la posibilidad de comparar la probabilidad de que un rasgo se adapte por azar con la probabilidad de que se adapte por selección natural. Se encontró que es posible la adaptación de un rasgo por azar (i.e., en un régimen donde predomina la deriva aleatoria) para rasgos extremadamente simples (i.e., para un bajo

número de dimensiones en el FGM), pero la probabilidad decrece drásticamente para rasgos de mayor complejidad. También se propone un método heurístico de prueba del efecto creativo de la selección natural. Dada la abstracción del modelo, sus resultados se vuelven difíciles de contrastar empíricamente, pero se proponen algunas líneas de razonamiento que tienden a promover futuros métodos de prueba empírica del efecto creativo de la selección natural. En el Apéndice B se hizo una revisión histórica y un intento de formalización de los alcances explicativos de la teoría de la selección natural en términos probabilísticos.

Un tercer problema abordado en esta tesis corresponde a lo que se ha considerado un Principio de la Evolución Molecular, a saber, que "las moléculas o partes de moléculas funcionalmente más importantes evolucionan más lento que las menos importantes". Recientes estudios empíricos que han buscado este patrón en proteínas, midiendo la importancia funcional de éstas según su grado de pleiotropía, no han hallado este patrón o se han encontrado resultados equívocos. En el Capítulo 3 se desarrolló un modelo donde se simula la evolución molecular de genes con distinto grado de pleiotropía, considerando su efecto fenotípico en el FGM. Los resultados mostraron que la evidencia empíricamente encontrada podría explicarse tanto por un modelo casi-neutral como por un modelo selectivo cuando se contemplan las consecuencias de la complejidad de los efectos fenotípicos de las mutaciones sobre la tasa de sustitución genética. La versatilidad del FGM así como su énfasis en el fenotipo nos ha permitido abordar tres fundamentales e interrelacionados procesos evolutivos, aún cuando se centren en distintos niveles de organización biológica, abarcando organismos (Capítulo 1), rasgos (Capítulo 2) y genes (Capítulo 3).

ABSTRACT

Thesis titled: "Models of molecular evolution in the Fisher's phenotypic space: general models of molecular evolution, the creative role of natural selection, and the role of gene pleiotropy on the rate of molecular evolution"

Evolutionary models at the molecular level usually do not contemplate the phenotypic effect of mutations, in spite of their overall effect on fitness. The Fisher's geometrical model (FGM) is an exception and in the last fifteen years it has been continued being developed and there is a increasing number of publications that utilize it to targe several problems of evolutionary biology (see Introduction). In this thesis the FGM is used to confront three problems connected with three classical issues of evolutionary theory and that are unsolved up to nowadays.

The first problem undertakes the aims of general theories of molecular evolution, where the major questions are the following: what is the predominant evolutionary force in evolution (mutation, selection, drift), what kind of mutations are substituted (neutrals, nearly-neutrals, advantageous, deleterious), and what is the relationship between the rate of molecular evolution and population size (independent, increasing or decreasing). The Chapter 1 propose a model of molecular evolution in the FGM to characterize both a balanced nearly-neutral evolutionary process in a stable environment, and a selective process with environmental variability. Results showed that the balanced model predicts an evolutionary rate that decrease with increasing population size, similarly to other nearly-neutral models, but it discards that the compensatory mutations explain the high rate of

advantageous substitutions currently found in DNA studies in many species, as it has been assumed previously. In turn, the selection model, predicts the independence of the evolutionary rate with population size, in agreement with recent empirical studies in *Drosophila* which has been difficult to interpret. In both models, we found that there are a tight relationship between key parameters for the molecular evolution, due to factors that are interpretable in biological terms, and that support a rethought of the arbitrariness wherewith usually are determined the parameters in other molecular evolutionary models. In order to carry out the simulations it was necessary the melioration of previous methods of multidimensional random vectors generation (Appendix A).

The second problem refers to the creative role of natural selection, a debate that beginning after Darwin's publication of the *Origin of Species* in 1859 and that continues until now. It has been questioned if natural selection can explain the origin of organismal traits in nature, but the debate, either pro or contra, it has been mainly given in metaphorical terms and with scarce or nil empirical support. For these reasons, we have reviewed the empirical tests available to date that are aimed to prove adaptations in natural populations (Chapter 2, Section 2). We found that currently there is no methodological test with the goal of proving the role of natural selection on trait formation, in spite that recent combined approximations have been pointed in that direction. In the Section 2 of Chapter 2, we introduced a model that compares the probability that a given trait adapts by chance and the probability that it adapts by natural selection. We found that the adaptation of a trait by chance (i.e., in a regime where random drift predominate) is possible for extremely simple traits (i.e., for a low number of dimensions in the FGM), but the probability decrease drastically for more complex traits. Furthermore, we propose a heuristic method to prove

the creative effect of natural selection. Given the abstraction of the model, its results are difficult to contrast empirically, but we propose some lines of thought that promote future empirical test methods for the creative role of natural selection. In the Appendix B we made an historical review and an attempt to formalize the explanatory scopes of the natural selection theory in probabilistic terms.

A third problem faced in this thesis is about what is considered a Principle of Molecular Evolution, that is the assertion that: "the functionally more important molecules or parts of molecules evolve more rapidly than the less ones". Recent studies have searched this pattern in proteins, measuring their functional importance as the degree of pleiotropy, but up to now no pattern has been founded or studies have founded ambiguous results. In the Chapter 3 we developed a model where the molecular evolution of proteins with different gene pleiotropy, considering its phenotypic effect in the FGM. The results showed that the empirical evidence founded could be explained both by a nearly-neutral and by a selection model, if the complex phenotypic consequences of mutations are taken into account on the genetic substitution rate. The FGM's versatility and emphasis on the phenotype enabled to undertake three fundamental and interrelated evolutionary process, in spite of they are centered in different levels of organization, including organisms (Chapter 1), traits (Chapter 2) and genes (Chapter 3).

INTRODUCCIÓN: EL MODELO GEOMÉTRICO DE FISHER

El modelo geométrico propuesto por Ronald Fisher en 1930 (FGM), fue originalmente utilizado por Fisher como argumento en contra de la importancia de las mutaciones de gran efecto fenotípico en la evolución (Orr 2005b). El modelo consiste en un espacio cartesiano n-dimensional cuyos ejes representan los posibles valores de rasgos fenotípicos cuantitativos de los organismos. El origen del sistema de coordenadas representa la combinación de valores fenotípicos en que la adecuación biológica de los organismos es máxima (i.e. la probabilidad de éxito reproductivo de los organismos con esa combinación de valores fenotípicos es mayor a cualquier otra combinación alternativa). Fisher supuso que si los organismos de una población no tienen la combinación óptima, por ejemplo, debido a un repentino cambio ambiental que modifica el fenotipo óptimo, entonces la población se encontraría a cierta distancia del punto óptimo, y las mutaciones, que modifican el fenotipo de los organismos, pueden representarse como vectores con diferentes direcciones (Fig. 1). Un resultado clásico de Fisher (1930) es que cuando las mutaciones son extremadamente pequeñas, la probabilidad de que sean favorables tiende a ser 0.5 y que a medida que aumenta su tamaño, la probabilidad disminuye exponencialmente (relación también llamada "ley de Fisher", Budd 2006). Este resultado fue citado por varias décadas como el argumento teórico favorito en apoyo del micromutacionismo (Orr & Coyne 1992), pero fue poco desarrollado (con ciertas

excepciones; véase por ejemplo Kimura 1983, caps. 6-7 y Leigh 1987) hasta los años 90 cuando comienza un aumento notorio en su uso y perfeccionamiento (Fig. 2).

El FGM es considerado, junto con los modelos en el espacio de secuencias (Gillespie 1984, 1991, Kauffman 1993), uno de los principales modelos para estudiar la genética de la adaptación (Orr 2005a, 2005b), y de hecho algunos de sus resultados han sido considerados particularmente iluminadores con respecto a la comprensión de la importancia de la complejidad de los organismos en la adaptación (Orr 2000, 2005b, Welch & Waxman 2003). Otro de los principales desarrollos del FGM ha sido el estudio de la carga fijada por deriva (*fixed drift load*) consistente en la adecuación biológica de equilibrio subóptima debido a la fijación de mutaciones deletéreas por efecto de la deriva aleatoria sobre poblaciones finitas (Hartl & Taubes 1998, Poon & Otto 2000, Sella & Hirsh 2005, Tenaillon et al. 2007, Sella 2009). Se ha demostrado que ésta depende sólo del número de dimensiones, el tamaño poblacional y la función de decaimiento de la superficie de adecuación biológica (véase Fig. 3). Otros usos del FGM incluyen el estudio de la distribución del tamaño de efectos mutacionales fijados durante la adaptación (Orr 1998, 1999, 2005a), la distribución de los coeficientes de selección de las mutaciones (Orr 2006, Martin & Lenormand 2008), el derretimiento genético (*genetic meltdown*) y el riesgo de extinción poblacional (Poon & Otto 2000, Whitlock 2000, Whitlock et al. 2003), la evolución molecular (Sella & Hirsh 2005, Gu 2007a, 2007b, Sella 2009), la evolución del desarrollo (Rice 1990), la evolución de la sexualidad (Barton 2001) y la genética del envejecimiento (Moorad and Promislow 2008).

El creciente interés por el desarrollo teórico del FGM (Fig. 2) probablemente no sólo se debe a la versatilidad de esta aproximación sino también a la posibilidad de asociar

resultados del FGM con aproximaciones experimentales y la contrastación de datos empíricos (Burch & Chao 1999, Haygood 2006, Martin & Lenormand 2006a, 2006b, Gu 2007b, Martin et al. 2007, Tenaillon et al. 2007). Además, la mayor parte de los estudios que han evaluado el efecto de relajar los supuestos aparentemente arbitrarios del FGM (como su simetría entre ejes, su geometría esférica, dependencia de distintas superficies de fitness, y otras), han llevado a la conclusión de que muchos resultados en el FGM son robustos con respecto a sus supuestos básicos (Rice 1990, Whitlock et al. 2003, Waxman & Welch 2005, Waxman 2006, Martin and Lenormand 2006a, Waxman 2007).

La versatilidad del modelo permite estudiar fenómenos evolutivos de diferente escala. Originalmente Fisher (1930) interpretó el modelo como una representación de un rasgo con diferentes aspectos fenotípicos, y el número de dimensiones como la complejidad del rasgo en cuestión (Orr 1999, Welch & Waxman 2003), interpretación que es mantenida por Orr (1999). La interpretación más usual es que el FGM representa los rasgos fenotípicos de organismos completos, y el número de dimensiones es interpretado como la complejidad de los organismos (e.g., Orr 1998, 2000, Welch & Waxman 2003, Martin & Lenormand 2006a, 2006b, Tenaillon et al. 2007). Más recientemente se ha interpretado el número de dimensiones como el número de rasgos fenotípicos afectados por las mutaciones en una proteína (pleiotropía génica), donde cada dimensión es considerada un "fenotipo molecular" (Gu 2007a, 2007b). El número de dimensiones ha sido estimado empíricamente para organismos completos (Tenaillon et al. 2007), y para proteínas (Gu 2007b), pero es dudoso que estos resultados sean robustos a los supuestos de los modelos utilizados (véase Capítulo 3).

Las distintas interpretaciones posibles del FGM nos han permitido abordar problemas de diferente escala en la biología evolutiva. En el Capítulo 1 se aborda el problema de las teorías generales de la evolución molecular, y el MGF se interpreta representando a los organismos completos (la manera más usual), de tal forma que las mutaciones representan las modificaciones genéticas con efecto fenotípico en cualquier parte del genoma de los organismos. En el Capítulo 2 se afronta el problema de la creatividad de la selección natural, generalmente entendida como la capacidad de la selección natural de formar rasgos complejos por la acumulación de sustituciones favorables en direcciones que llevan a la modificación coherente e integrada de los cambios fenotípicos provocados por dichas mutaciones. En este contexto, el FGM se interpreta representando a un rasgo, o posibles estados de un rasgo (la manera de Fisher 1930 y Orr 1999) y las mutaciones son interpretadas como aquellas que pueden ocurrir en cualquiera de los genes cuya modificación puede generar un efecto fenotípico sobre el rasgo. Por último, en el Capítulo 3 se trata el problema del papel de la pleiotropía sobre la tasa de evolución molecular. Aún cuando los biólogos evolutivos reconocieron tempranamente que genes únicos afectan múltiples rasgos (e.g., Dobzhansky & Holz 1943, Caspari 1952, Wright 1968), y existen en la actualidad diversas formas de estimar estos efectos (véase Capítulo 3), la pleiotropía ha sido largamente ignorada en los modelos tradicionales de la genética de poblaciones (Otto 2004). Además, la búsqueda de patrones que relacionen la tasa de evolución molecular con la pleiotropía de las proteínas ha llevado a poner en duda la aplicabilidad de uno de los principios de la evolución molecular a las proteínas y, con ello, a las teorías neutralistas que lo sustentan (Camps et al. 2007). Para afrontar este asunto se generó un modelo en el espacio geométrico de Fisher donde el número de dimensiones es una propiedad de las proteínas, es decir, se interpreta el FGM como representando los

rasgos de los organismos que son afectados por las mutaciones sobre un solo gen (la manera de Gu 2007a, 2007b).

Las interpretaciones del número de dimensiones del MGF como una propiedad de genes, de rasgos y de organismos no son inconsistentes entre sí, ni son interpretaciones incompatibles. Por el contrario, pueden ser complementarias. Un ejemplo de aproximación que relaciona dos de estos niveles (rasgo-organismo) es el trabajo de Welch y Waxman (2003), donde se estudia paralelamente la inferencia del número de rasgos modulares que tienen los organismos, y la evolución de los organismos completos, cuya dimensión dependerá del número de módulos y del número de dimensiones de cada módulo. En los tres niveles estudiados en esta tesis, la biología evolutiva enfrenta problemas que requieren tanto de la búsqueda de patrones experimentales como de modelos teóricos que permitan la comprensión y explicación de dichos patrones. En esta investigación se encontraron resultados teóricos que pueden aportar en el avance hacia la resolución de estos problemas.

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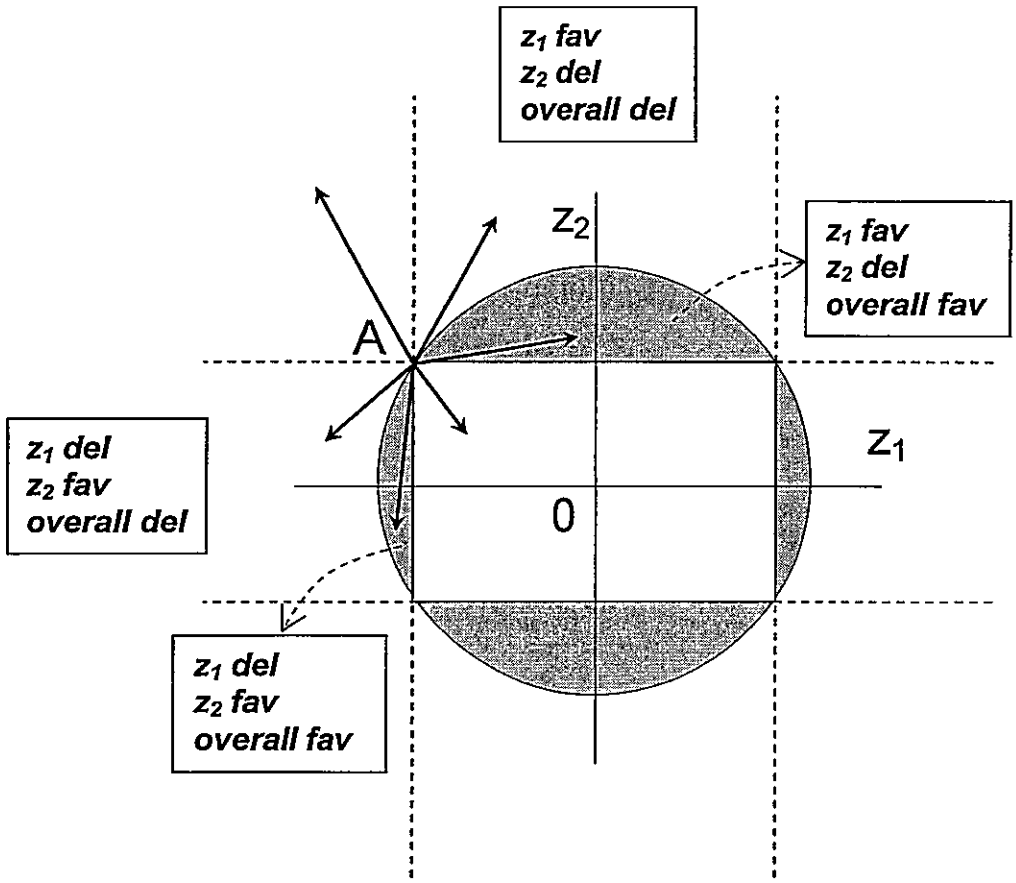
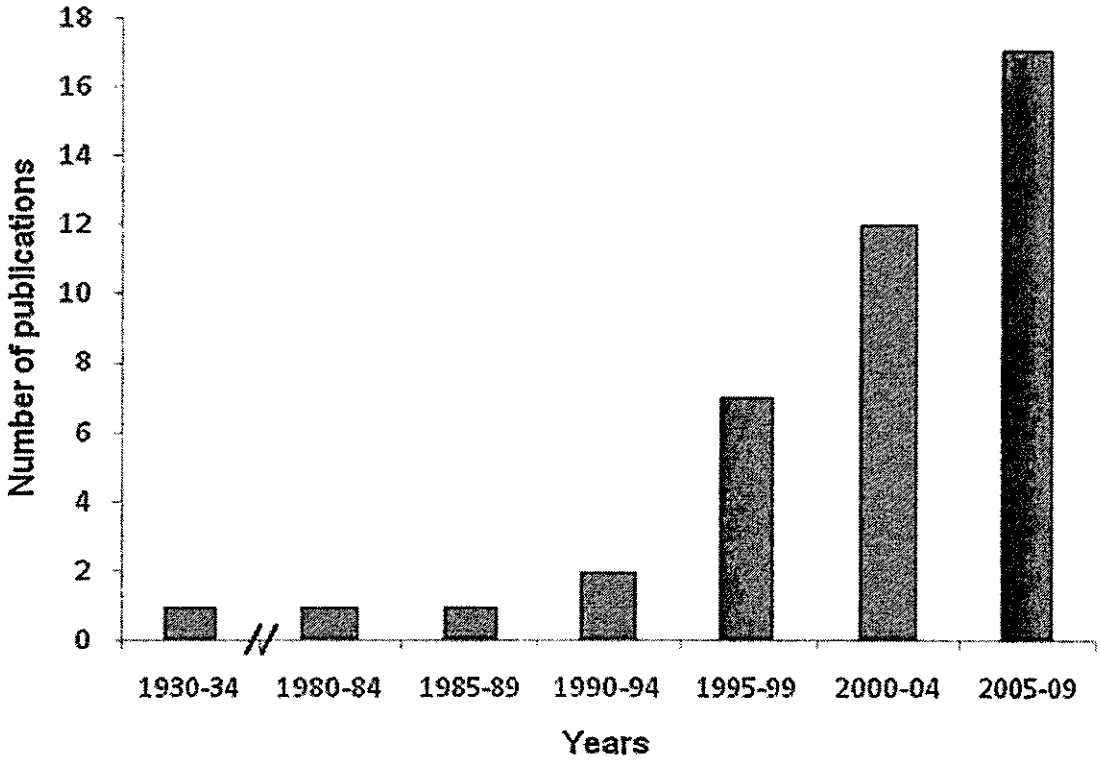


Figura 1: Tipos de mutaciones que pueden afectar el fenotipo de un organismo en el modelo geométrico de Fisher, representado en dos dimensiones. Las mutaciones que caen en el cuadrado blanco son beneficiosas para ambos rasgos. En cambio, las mutaciones que caen en la zona gris son beneficiosas (*ben*) para un rasgo pero deletéreas (*del*) para el otro, con un efecto neto beneficioso (*overall fav*) en la medida que caen dentro del círculo. Las mutaciones que caen fuera del círculo tienen un efecto neto deletéreo, pero pueden favorecer a un rasgo y desfavorecer a otro.

a)


Number of publications
18
16
14
12
10
8
6
4
2
0
1930-34
1980-84
1985-89
1990-94
1995-99
2000-04
2005-09
Years

b)

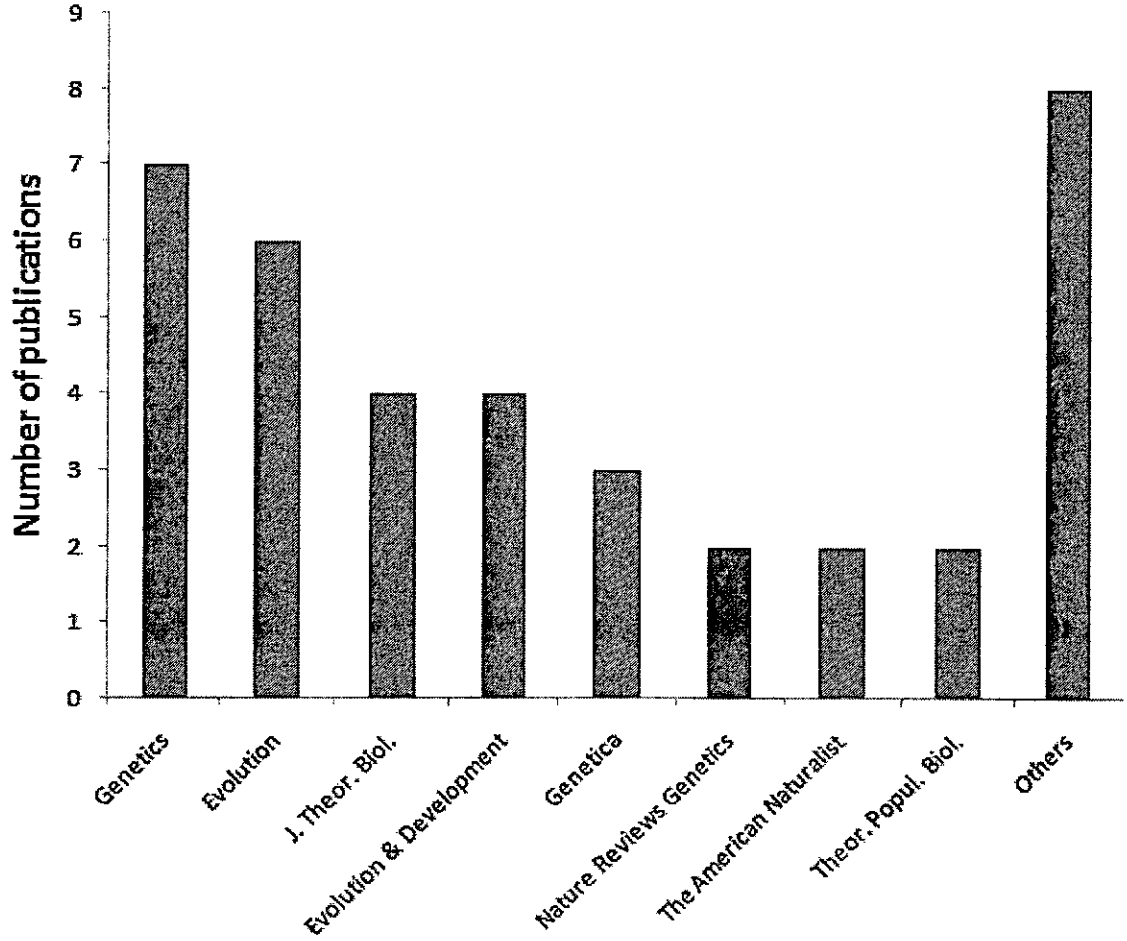

Number of publications
9
8
7
6
5
4
3
2
1
0
Genetics
Evolution
J. Theor. Biol.
Evolution & Development
Genetica
Nature Reviews Genetics
The American Naturalist
Theor. Popul. Biol.
Others

Figura 2: a) Número de publicaciones por año donde se desarrolla o analiza el modelo geométrico de Fisher. Resultado exhaustivo del total de publicaciones sobre el tema (41) excluyendo manuscritos no publicados disponibles en Internet. b) Distribución de los mismos artículos según la revista donde se publicó. En la categoría "others" se encuentran las revistas donde se ha publicado sólo un artículo, las cuales son *Nature, Mol. Ecol., PNAS, Mol. Biol. Evol., Plos One, Nature Genetics, Ann. Zool. Fennici.* y *Genet. Res. Camb.* La búsqueda se hizo a partir de las bases de datos de ISI Web of Science y Scholar Google.

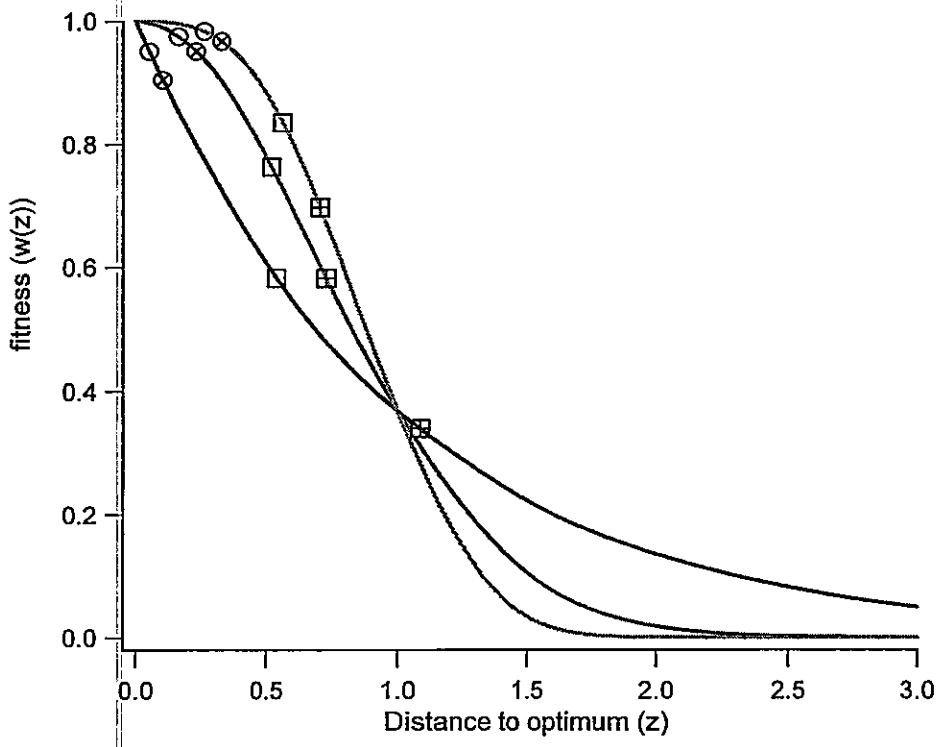


Figura 3: Las curvas representan superficies de adecuación biológica para distintos valores de distancia desde el óptimo. Desde la más oscura a la más clara, las curvas corresponden a $W(z)=e^{-z}$, $W(z)=e^{-z^2}$, $W(z)=e^{-z^3}$, respectivamente. Los círculos corresponden al fitness promedio de equilibrio para poblaciones de tamaño $N=100$ y los cuadrados, $N=10$. Marcadores abiertos correponden a número de dimensiones $n=10$ y los marcadores cruzados, a $n=20$.

CAPÍTULO 1

MODELOS GENERALES DE LA EVOLUCIÓN MOLECULAR EN EL ESPACIO GEOMÉTRICO DE FISHER

The nearly-neutral and selection theories of molecular evolution under the Fisher's geometrical framework: substitution rate, population size and complexity

ABSTRACT: The general theories of molecular evolution depend on arbitrary assumptions about the relative distribution and rate of advantageous, deleterious, neutral and nearly-neutral mutations. The Fisher's geometrical model (FGM) has been used to make distributions of mutations biologically interpretable. We explored a balanced steady state model with compensatory mutations in the FGM as a nearly-neutral model of molecular evolution. We also studied the process under random environmental fluctuations which is characterized by an interplay between adaptive processes and nearly neutral steady state processes. We found that the balanced steady state is a nearly-neutral process with a negative relationship between molecular evolutionary rate and population size but with different properties compared with previous balanced nearly neutral models, which have problematic properties like its narrow window of strength of selection where they work. Besides, the balanced steady state cannot explain the high rate of fixations driven by positive selection currently found in DNA sequences, contrary to what has been assumed previously. When temporal environmental fluctuations are incorporated, the process becomes a selection model where the complexity of organisms is a critical factor for the evolutionary rate. Key parameters of molecular evolution are linked by biological factors implying their coupling or even their locking, and then we showed that they cannot be fixed independently by arbitrary criteria, as is usually made.

KEY WORDS: molecular evolution, balanced steady state, fluctuating environment, population size, complexity, Fisher's geometrical model

INTRODUCTION

The nearly neutral theory of molecular evolution (Ohta & Kimura 1971, Ohta 1972, 1973, 1977, 1992, 1996), as is generally understood, affirms that a vast majority of amino acid substitutions are slightly deleterious; hence it was called the slightly deleterious mutation theory (Ohta & Kimura 1971, Ohta 1972, 1973, 1977, 1987, 1996, Kimura 1983, Gillespie 1995, 2004, Kreitman 1996). The original exponential shift model of Ohta (1977) was modified by Kimura (1979) whom proposed the gamma shift model to overcome the Ohta's previous assumptions that implies a too low rate of substitutions when population size increases. Gillespie (1994, 1995) uncovered that the later type of nearly neutral models, known as the house-of-cards or "fixed" models (Ohta & Tachida 1990, Tachida 1991) have a very different result than the slightly deleterious mutation theory prediction, because in these models only a half of substitutions are deleterious and the other half are advantageous (see also Tachida 1996, 2000). These models can be grouped in a different category that we will call the balanced steady state theory (Fig. 1). Nevertheless, these models were strongly criticized because the rate of substitutions ceases for middle size population (specifically for $2N\sigma_s > 4$, where σ_s is the standard deviation of selection coefficients, and N is the effective population size), that is, except for a narrow window they produce even lower rates of substitutions than those observed by the original exponential shift model (Gillespie 1994, 1995, 1999, Ohta & Gillespie 1996). Thus, the nearly neutral theory continued emphasizing the substitution of slightly deleterious mutations (Ohta 1992, 1996, 2007, Gillespie 1995, 2004, Eyre-Walker et al. 2002, Sella & Hirsh 2005, Gu 2007a, 2007b). Selection models, where natural selection rather than genetic drift is the main force causing fixations, commonly depend on fluctuating environments that are required to continue the

evolution and could explain some of the empirical phenomena found in molecular data (Ohta & Gillespie 1996). Nevertheless, it has been recognized that there is a lack of a general model of molecular evolution that can account for all major molecular phenomenology (Ohta & Gillespie 1996, Kreitman 1996).

On the other hand, theoretical studies of molecular evolution assume predefined distributions of selection coefficients of mutants (e.g., Ohta 1973, 1977, Kimura 1979, Ohta & Tachida 1990, Gillespie 1993, 1994). Thus the rate and proportion of different types of mutations is only dependent of the population size and some parameter of the selection coefficients distribution (typically $\overline{s}$ and σ_s, the mean and the standard deviation of selection coefficients, respectively). Nevertheless the distribution of mutant selection coefficients is determined by the operation of the evolutionary dynamic and therefore it cannot be assumed a priori (Sella & Hirsh 2005). Moreover, choosing a specified distribution is somewhat *ad hoc* because it lacks a clear-cut biological interpretation (Gu 2007b). The FGM (Fisher 1930) has been used to make distributions of mutations biologically interpretable (Martin & Lenormand 2006, 2008, Gu 2007a, 2007b). Given a distribution of mutation sizes, in the FGM the distribution of mutant selection coefficients is determined for geometrical relations between the number of traits of organisms and the distance to an optimum trait combination. In turn, this distance depends on the environmental changes (Orr 1998, 1999, 2000) and the fixed drift load of the population (Hartl & Taubes 1998, Poon & Otto 2000, Sella & Hirsh 2005, Tenaillon et al. 2007, Sella 2009), which allows exploring relationships between parameters that could be linked to biologically interpretable factors and should not be independently specified by arbitrary criteria. For example, Gu (2007a, 2007b) used the FGM to model the slightly deleterious

mutation theory under the shift models' framework, founding a natural explanation for the Kimura's gamma function of selection coefficients which would be related to the number of phenotypic dimensions influenced by the mutations on a gene (gene pleiotropy). Thus, Gu found that the gamma distribution of Kimura corresponds to $n=1$ phenotypic dimensions and the exponential distribution of Ohta corresponds to $n=2$ (Gu 2007a). Gu's work also allows the study of the relationship between molecular evolution and population size in the FGM. However, the assumptions of Gu's model in the FGM inherit the problems of the original shift models, which were strongly criticized because its biologically unreasonable assumptions (Tachida 1991, Ohta 1992, Gillespie 1995, Ohta & Gillespie 1996). For instance, shift models require that all mutations be deleterious. Thus, when a deleterious mutation gets fixed, all subsequent mutations must be less fit than the fixed mutation. Shift models allow making simplifying assumptions that were used by the Gu's work to make the problem analytically tractable. However, more realistic assumptions can be faced with computer simulations (as in the house-of-cards model). We relaxed the assumptions of Gu's model in the FGM and developed a model that support a balanced steady state theory of molecular evolution, which we call balanced geometric model (BGM). The steady state in the FGM has been understood as a nearly-neutral evolutionary process (Hartl & Taubes 1996, Sella & Hirsh 2005, Sella 2009) and it is in some aspects similar to the house-of-cards nearly neutral model (Ohta & Tachida 1990, Tachida 1991, 1996). We explored the possible interpretations and evolutionary consequences of this model through simulations in the Fisher's geometrical framework both in a balanced steady state (the BGM) as well as in the interplay between adaptive processes and balanced steady states in a randomly fluctuating environment, henceforth the variable geometrical model (VGM). We found that when molecular evolution, both in a fluctuating environment and in

the steady state, is biologically interpreted in the FGM, it does not enable the arbitrary independent specifications of a number evolutionarily relevant parameters because they are locked in relationships dependent on biologically interpretable factors. Also, we found other differences with previous nearly neutral models and previous expectations about the balanced steady state process that are specified below.

THE MODEL

Assumptions

Given their difficult analytical tractability, models where the population fitness fluctuates as a result of mutant fixations are analyzed by computer simulations (Ohta & Tachida 1990, Tachida 1991, 1996, 2000, Gillespie 1995). We simulated asexual populations under weak mutation ($Nu << 1$, where u is the genomic mutation rate), thus the evolutionary process is depicted as a succession of fixations and neglects the effects of polymorphisms. The FGM represents a population as a point in a n-dimensional space of states, where each axis represent a different organismal phenotypic trait and the origin of coordinates represents the optimum state of a population given a specific environmental condition (Orr 1998, 2000, Welch & Waxman 2003). Mutations are represented as random vectors uniformly distributed in this hyperspace. Vectors falling nearer to the optimum are advantageous and those pointing away from the optimum are deleterious, and its selection coefficients are determined by a Gaussian fitness function centered in the optimum (which, without loss of generality, takes the value 1). Environmental fluctuations are represented as optimum shifts (Barton 2001, Gu 2007b). Differently to the shift models, when a mutation is fixed the

phenotype of individuals in the population is modified to the new value of fitness, thus the subsequent mutations start from the new phenotypic state.

Usually the evolutionary process in the FGM is modeled in a bout of adaptation after a recent sudden environmental shift of the optimum (Orr 1998, 1999, 2000, Welch & Waxman 2003), but the fate of an adaptive bout is to reach a plateau (Silander et al. 2007), which is characterized by a balanced steady state where the molecular evolution does not stop (Hartl & Taubes 1996). Properties of the balanced steady state have been studied maintaining a fixed optimum (Hartl & Taubes 1998, Poon & Otto 2000, Sella & Hirsh 2005, Tenaillon et al. 2007, Sella 2009). In the VGM we modeled temporally fluctuating random optimum shifts, thus the evolutionary process is an alternation between adaptive bouts and balanced steady states that will be determined by the variability of the environmental changes.

We studied the molecular evolution as the ratio between the rate of substitutions and the rate of mutations (k/u), that is usually measured by the ratio between synonymous and non synonymous substitution rates (d_N/d_S), under the assumption that synonymous substitutions are almost neutral. Differently to Gu (2007a, 2007b) we utilized a top-down approach to the random vectors generation (Poon & Otto 2000). That is, we specified the distribution of total mutational length and left unspecified the component distributions along each axis. Thus, contrary to Gu (2007a, 2007b) a change in the number of dimensions does not affect the total length of the mutation's effects. We follow Gu's bottom-up approach to the random shifts of optimum values, but corrected the amplitudes of shifts in a manner that amplitudes be equal for different number of dimensions. The distribution used for mutational magnitudes is uniform, which implies that in each axis the distribution of

effects is leptokurtic, which corresponds with the empirical evidence (Keightley 1994; Lynch et al. 1999).

Simulations

Simulations were made with Monte Carlo methods where random vectors are generated with a uniform distribution of vector magnitudes (from 0 to r) (following Kimura 1983, Orr 1998). These changes can be fixed according to the probability function $p(N,s)=\frac{1-e^{-2s}}{1-e^{-2Ns}}$, where N is the effective population size and s the selection coefficient of the mutation (Crow & Kimura 1970).

Fitness values were determined by the Gaussian function $w(z)=e^{-\frac{z^2}{2}}$, where z is the distance to the optimum point. Selection coefficients are defined as $s=\frac{w_{mut}-w^+}{w^+}$, where w_{mut} is the fitness of the mutant and w^+ is the fitness of the wild-type. We obtained the ratio between substitution rate and mutation rate (k/u) for different conditions, varying complexity (number of dimensions), size of mutations (vector length), population size, amplitude and variability of optimum shifts.

Random environmental shifts were simulated as a Poisson process such that $\nu \sim f(\nu,\lambda)=\frac{e^{-\lambda}\lambda^{\nu}}{\nu!}$, where ν and λ are the number and the expected number of changes in a time interval, respectively. Time intervals between consecutive changes (t) followed an exponential distribution $t \sim f(t,\tau)=e^{-\frac{1}{\tau}t}$, where $\tau=1/\lambda$ is the expected time between environmental changes (pseudo-random numbers were obtained using *expnoise* function

available in IgorPro, WaveMetrics, Lake Oswego, OR). In order to compare results between different population sizes in variable environments we set $\tau \propto N$. The amplitudes of environmental changes were calculated as $a(z_1,...,z_n)=\frac{\sqrt{z_1^2+...+z_n^2}}{\sqrt{n}}$ where z_i are the coordinates of the new optimum which were chosen from a Gaussian distribution centered in the origin of the Cartesian n-dimensional space, i.e. $z_i \sim f(\sigma_a)=e^{\frac{z_i^2}{2\sigma_a^2}}$, where σ_a represents the standard deviation of amplitudes of environmental changes (we used *gnoise* function available in IgorPro, WaveMetrics, Lake Oswego, OR).

Strictly advantageous substitutions are defined as substitutions that accomplish $s > 1/N$, i.e. advantageous substitutions fixed by positive selection; effectively neutral substitutions are defined as $|s|<1/N$, i.e. substitutions fixed mainly by random drift; and strictly deleterious substitutions are defined as $s<-1/N$, i.e. deleterious substitutions fixed by drift in spite of its strong negative selection against them.

RESULTS

In Figure 2 we show a trial of the substitution process used for simulations with random environmental variability (the VGM). After an optimum shift, the population suffers a burst of adaptive substitutions (Fig. 2c) until reaching a balanced steady state (Fig. 2b). In the balanced steady state, the population remains around a sub-optimum equilibrium fitness that is lower for lower population size and where a fluctuating substitution process occurs (Fig. 2b). In the BGM the proportion between the number of advantageous ($s>0$) and deleterious ($s<0$) mutations depends on the size of mutations (Fig. 3, left column) with a

leptokurtic distribution skewed toward more negative values for higher mutation sizes. The proportion between advantageous and deleterious mutations tends to a maximum value of 0.5 for smaller mutation sizes (Fig. 3c, left column). The distribution of selection coefficients of substitutions (Fig. 3, right column) follows a leptokurtic but symmetric distribution independently of the mutation size. That is, the proportion of advantageous substitutions is always 0.5. The mean ($\overline{s}$) and standard deviation (σ_s) of selection coefficients of mutations were calculated according to different mutation sizes, both for steady state (BGM) and environmental fluctuating evolutionary processes (VGM) (Fig. 4). The mean selection coefficient decreases while the standard deviation increases with the increasing of mutation size under all conditions. The curves are clearly differentiated for lower population sizes (for $N = 10$ and $N = 100$), but they are very similar for higher population sizes ($N = 100$, 1000 and 10,000). Curves are only slightly sensible to other parameters as the number of dimensions and the environmental variability (Fig. 4).

We found a negative relationship between total and effectively neutral evolutionary rate and population size in stable environments (Fig. 5). The decrease is stronger for higher mutation sizes (Fig. 5). Effectively advantageous and deleterious substitution rates are equal and decrease with increasing population sizes for higher mutation sizes (Fig. 5a,b), have a maximum for intermediate sizes (Fig.5c), and increase for small mutation sizes (Fig. 5d). With environmental variability the results are more complex (Fig. 6). The total evolutionary rate decreases with the increasing of population size with an exception for small mutation sizes and high number of dimensions (Fig. 6d). The decreasing of total substitution rate reaches a plateau for low population size when mutation size is high (Fig. 6a, b). The value of this plateau, where the total substitution rate maintains equal for

different population sizes, is higher for lower mutation sizes. Effectively advantageous substitution rate increases with population size, and the increasing is stronger for lower mutation size, however the critical population size where advantageous rate exceeds the effectively neutral rate is higher for lower mutation sizes (Fig. 6).

DISCUSSION

The BGM showed similarities with the house-of-cards or "fixed" model of molecular evolution (Ohta & Tachida 1990, Tachida 1991), where evolution is an alternating process with a half of substitutions being advantageous and the other half being deleterious (Fig. 3) (Tachida 1991, Gillespie 1994, 1995, Sella & Hirsh 2005). Nevertheless, in the house-of-cards model the substitution rate has a concave function with the strength of selection ($2N\sigma_s$) and stops when $2N\sigma_s > 4$ (Tachida 1991, Gillespie 1994, 1995, Ohta & Gillespie 1996). Contrarily, in our model the relationship between substitution rate and population size (and thus the strength of selection) is convex (as in the shifting models, Gillespie 1994, 1995) and evolution does not stop even for $2N\sigma_s \approx 260$ (e.g., in Fig. 5a $\sigma_s \approx 0.13$, and when N=1000, $k/u \approx 0.05$). Tachida (1996) showed that in the house-of-cards model substitutions continue to occur even when $2N\sigma_s$ is 20 if the distribution of coefficient of mutations is uniform. Nevertheless this assumption is not realistic according to the current data that show leptokurtic selection coefficient distributions (Keightley 1994; Lynch et al. 1999), as in our BGM. This difference could be explained because in the FGM it is possible overshoot the optimum value, i.e. mutations directed to the highest fitness in the FGM could decrease the fitness because it could overshoot the optimum value. However in the

house-of-cards model this is impossible and all mutations directed toward the direction of higher fitness confer higher fitness if they are fixed, because advantageous mutations can take unlimited positive selection coefficients. The consequence is that the evolution in the house-of-cards models tends to stop because the pressure toward higher fitness decreases the number of possible ulterior advantageous mutations.

There are few theoretical models in evolutionary biology in which compensatory mutations are explicitly incorporated (Poon et al. 2005) as in the BGM and the VGM. Consequently, the evolutionary role of compensatory mutations is not completely understood. For example, it has been suggested that the current evidence of a high rate of advantageous mutations fixed by positive selection, around 50% or more (Fay et al. 2002, Bierne et al. 2004, Eyre-Walker 2006, Bachtrog 2008), could be explained as the effect of compensatory mutations (Kondrashov et al. 2002, DePristo et al. 2006, Pal et al. 2006, Camps et al. 2007). This idea was proposed in the FGM by Hartl & Taubes (1996) whom maintained that in the steady state there is "selection without adaptation", i.e., positive selection but only upholding the *status quo* in a balance between deleterious mutations and posterior advantageous compensatory mutations. In contrast with this assumption, we found that the proportion of advantageous mutations fixed by positive selection (i.e. strictly advantageous mutations) is very low in the steady state (Fig. 5). The explanation for this result is that compensatory mutations come after one or a very small number of deleterious mutations fixed by drift, and then both are in the same order of magnitude, i.e. both are mainly effectively neutral (Fig. 5). In other words, because in the BGM no much more than 50% of substitutions are advantageous and the distribution of selection coefficients of substitutions is leptokurtic (Fig. 3), necessarily a small proportion of advantageous

substitutions ($s > 0$) could be strictly advantageous ($s > 1/N$). It would be possible that if compensatory mutations are very rare, in average several deleterious substitutions could be fixed before than an advantageous mutation compensates the previous effect of the deleterious ones. In this case, a higher proportion of compensatory substitutions could be of higher size and strictly advantageous. On the contrary, in our model we assume a high rate of compensatory mutations, which is in agreement with current studies (Poon & Otto 2000, Whitlock et al. 2003, Ponn et al. 2005). For example, Ponn et al.'s (2005) study in virus, prokaryote, and eukaryote revealed that there are in average 11.8 compensatory mutations per deleterious mutation. Our conclusions seem robust under this panorama.

Both the slightly deleterious mutation and the balanced steady state theories (Fig. 1) have been considered within the "nearly neutral theory". In turn, the distinction between the nearly neutral models and selection models is not quite clear (Ohta 1996, Kreitman 1996, Ohta & Gillespie 1996). The main difference between the nearly neutral and selection theories is that the former predicts a negative relationship between evolution and population size whereas the latter predicts the contrary one (Ohta 1996). The role of population size in population genetic models of molecular evolution is examined by Gillespie (1999), who has defined three domains according to the relation between substitution rate and population size. In the Ohta's domain, the rate of substitution decreases with increasing population size, while in Kimura's domain, the rate of substitution remains close to the mutation rate and in the Darwin's domain, the rate of substitution increases with increasing population size. The inverse relationship between the substitution rate and population size is verified in our simulations (Fig. 5) (see also Gu 2007a), thus our BGM falls under the Ohta's domain. Because this inverse relationship is essential to explain the protein molecular clock under

the nearly neutral theory (Ohta 1992) the BGM can be understood as a nearly neutral model too. However, it is important to distinguish the BGM from the slightly deleterious mutation theory (Fig. 1), which is the model most commonly associated with the nearly neutral theory (Gillespie 1995, 2004, Ohta 1996). The differences between the slightly deleterious mutation models and the BGM are important, both for mutation and substitution hypothises, and they are the following: i) the mutation assumptions of BGM involve a higher fraction of advantageous (mainly compensatory) mutations than the slightly deleterious mutation models; and ii) BGM's predictions imply a much higher fraction of advantageous substitutions than the slightly deleterious mutation models, so that, as a minimum, BGM involves a 50% of advantageous substitutions (Fig. 1).

In the BGM the total evolutionary rate is determined mainly by the effectively neutral mutations, and is higher to lower mutation sizes (Fig. 5), which is consistent with the decreasing of the mean selection coefficients of larger mutations (Fig. 4a). When the rate of strictly advantageous and strictly deleterious substitutions are described apart, we found that they decrease with population size for higher mutation sizes (Fig. 5a,b), have a maximum for intermediate sizes (Fig. 5c), and increase with the population size for lower mutation sizes (Fig. 5d). The decreasing of the evolutionary rate with population size is the commonly expected behavior under the nearly neutral framework, because higher population sizes implie strong selection against the deleterious mutations, decreasing the rate of deleterious mutations and then of advantageous compensatory mutations. Nevertheless, the increasing rate for lower mutation sizes (Fig. 5d) is not predicted by the nearly-neutral models, because with small mutation sizes, the BGM implies that a high proportion of nearly neutral mutations (50%) are advantageous (Fig. 3c). Under this

situation, the distribution of coefficient of selection is symmetrical (Fig. 3c) and the increasing of population size has the effect of increase the strength of selection equally for advantageous and deleterious mutations. Because the probability of fixation is higher for advantageous than deleterious mutations, the effect of the increasing of the strength of selection is the increasing of the rate of strictly advantageous mutations with the respective balance of strictly deleterious ones (Fig. 5d).

Contrary to previous studies of the FGM that have been focused separately in the adaptive process (Orr 1998, 1999, 2005, Welch & Waxman 2003, Griswold & Whitlock 2003) or in the steady state (Hartl & Taubes 1998, Poon & Otto 2000, Sella & Hirsh 2005, Tenaillon et al. 2007, Sella 2009), the VGM is a model where there is an interplay between adaptive and balanced steady state processes (Fig. 2). Generally, selection models of molecular evolution assume environmental changes (Gillespie 1993, Ohta & Gillespie 1996), as it is observed, for example, in the mutational landscape model (Gillespie 1984, 1991), NK model (Kauffman 1993), TIM model (Takahata et al. 1975) and SAS-CFF model (Gillespie 1991). The reason is that populations likely evolve to the point where most mutations are deleterious through the substitution of advantageous mutations. The idea that permanent advantageous fixation by positive selection could occurs without optimum shifts probably becomes from early findings in the vertebrate major histocompatibility complex and co-evolutionary processes of pathogens that erroneously are taken as model for the general evolution of proteins (see Hughes 2007). Thus, a more general molecular evolutionary model would predict that when all mutationally accessible advantageous alleles are exhausted, the majority of newly arising mutations will be deleterious (Gillespie 1994) or nearly-neutral (Hartl & Taubes 1996). Accordingly, in the

VGM the evolutionary rate increases with temporal environmental fluctuations (Fig. 6) compared with the rate without environmental fluctuations but with the same mutation sizes (Fig. 5). Given that in the VGM populations reach to dynamic (i.e. it does not stop) steady states after the adaptive bouts, evolutionary rate does not stop without environmental fluctuations (contrary to other models, Gillespie & Ohta 1996). Nevertheless, whether compensatory mutations cannot explain the repeated substitutions by positive selection, and because positive selection tends to stop after some steps (Hughes 2007), the environmental shifts assumption is the only remaining reasonable hypothesis to maintain the selection model at the molecular level.

The higher evolutionary rate in the VGM compared with the BGM is evidently due to an increase of strictly advantageous substitution rate (Fig. 6). Interestingly, this increasing is higher for small mutation sizes, attaining $k/u > 1$ under some conditions. Paradoxically, this does not imply that for smaller mutation size the evolutionary process becomes more influenced by natural selection. In fact, the rate of strictly advantageous substitutions exceed the effectively neutral with larger mutation sizes even for low population size (Fig. 6a,b) but it did not occur with smaller mutation sizes (Fig. 6d). The increasing of the evolutionary rate for lower mutation sizes is because the adaptive process needs a higher number of advantageous substitutions to reach the steady state. Interestingly, the evolutionary rate attain a plateau that does not depend on the population size but on the complexity (number of dimensions) of organisms (Fig. 6a,b). For higher complexity of organisms the evolutionary rate increases, which can be understood as a consequence of the Orr's (2000) "cost of complexity" (see Chapter 3). The independence of the mutation rate with the population size in the plateau is due to the increasing of strictly advantageous

substitutions is accurately balanced by the decreasing of effectively neutral substitutions (Fig. 6). As effectively neutral substitution rate decrease to zero, the strictly advantageous increase to a plateau. This is consistent with recent evidence about the rate of strictly advantageous substitution rate that seems to be independent of population size (Bachtrog 2008). Bachtrog (2008) found only a slightly higher rate of strictly advantageous substitutions in *D. melanogaster* than in *D. miranda*, in spite of the latter having an effective population size 5-fold smaller than the former.

As is expectable, the increasing of environmental amplitude has an equivalent effect on the evolutionary rate than the increasing of environmental variability (not shown), but biologically these phenomena are not completely equivalent. Indeed, an important assumption of the VGM simulations is that populations do not go to extinct due to environmental fluctuations. In fact, populations cannot support a too high level of load (i.e. suboptimum fitness value due to an environmental shift of high amplitude) (Haldane 1957). For example, in our simulations the mean load due to environmental fluctuations is $L = 0.3$ (i.e. $w = 0.7$, for $\sigma_a = 0.85$). That is in the order of the cost assumed by Ewens (2004), but Haldane (1957) assumed a load of 0.1 based on human data. This value is probably conservative and depends on the population density (Nunney 2003). Overall, populations with a high reproductive excess could bearing the fitness decrease due to environmental changes (Nunney 2003, Ewens 2004). Other assumption of our model where the strength of the environmental change is important was that the fitness surface is Gaussian. This is an assumption usually justified because when population is close to the optimum, a Gaussian fitness function is a good local approximation for many arbitrary fitness functions (Lande

1980), however it could be less accurate under strong environmental change where the population is not so near to the optimum (Martin & Lenormand 2006a).

Besides the linked parameters founded in the BGM and VGM other interesting coupling were found. For example, for both the BGM and the VGM the magnitude of the coefficient of variation of selection coefficients is near to one when $N \geq 100$ (Fig. 4b), that is, if the mean of selection coefficients decreases, the standard deviation increases nearly in the same proportion. A plus point of the FGM is that allows making some of the distributions used in molecular evolution biologically interpretable (Sella & Hirsh 2005, Martin & Lenormand 2006, 2008, Gu 2007a, 2007b). *A priori* assumption of a particular distribution of mutant selection coefficients is inappropriate because the distribution determined by the evolutionary dynamic will differ in important ways from distributions assumed *a priori* (Sella & Hirsh 2005), but also because the principal parameters assumed in the general models are crucial by themselves for other biologically relevant issues. For example, the proportion of deleterious to advantageous mutations is crucial to estimate probability of extinction of populations and it is frequently fixed arbitrarily in the models due to the lack of other theoretical or empirical criteria to determine it (Whitlock 2000, Whitlock et al. 2003).

Several parameters are linked in the BGM. The proportion of advantageous and deleterious mutations around a fixed point from an optimum is locked, i.e., it cannot take arbitrarily independent values. It yields a minimum value of 0.5 when the mutation size tends to zero (Fig. 3c, as argued by Fisher 1930). When the mutation size is small the limiting factor for the selection coefficient of deleterious and advantageous mutations is the size of mutations (Fig. 3c), and a high proportion (50%) of nearly neutral mutations are

advantageous (contrary to the Ohta's (1977, 1992) assumptions, but corresponding with the Fisher's (1930) classical result, see Gillespie 1995). In contrast, when the mutation size increases, the magnitude of selection coefficients of deleterious mutations increases, while selection coefficients of advantageous mutations do not (Fig. 3a). In this situation a higher proportion of nearly-neutral mutations are deleterious (>50%), as in the assumptions of Ohta's models. For higher mutation sizes, the maximum value of the selection coefficient of an advantageous mutation is the fixed drift load of the individuals. An approximate value for the load in a Gaussian fitness surface is (Poon & Otto 2000):

$$L = \frac{n}{n+4N}$$

(This formula is approximate to the exact formula proposed by Tenaillon et al. (2007), but we use it for simplicity). When load is lower than the range of effective neutrality (i.e. $L < 1/N$) advantageous mutations must be effectively neutral, and advantageous mutations are not fixed by natural selection. This condition meets only when the number of dimensions is:

$$n < \frac{2N}{N-1},$$

which has maximum of $n = 4$ (when $N = 2$) and generally is fulfilled only for one or two dimensions. This implies that except for very simple organisms ($n = 1$ or 2), the limiting factor for the size of advantageous substitutions is mainly given by the selection coefficient of previous deleterious mutations and not by the distance to the optimum (the fixed load).

Besides the linked parameters described previously (the size of advantageous and deleterious mutations, the mutation size, the fixed drift load, the complexity and the

population size), other parameters, such as $\overline{s}$ and σ_s are coupled in the BGM (Fig. 4). As a matter of fact, we have also made some *a priori* assumptions, as the fitness profile and the distribution of mutations. Although these assumptions seem more biologically realistic than previously thought (Martin & Lenormand 2006b), it would be important to study the robustness of our results with respect to these assumptions. Simulations have been developed and preliminary results indicate that three of the conclusions of this work are very robust (not shown). First, compensatory substitutions cannot take arbitrary values and necessarily a low proportion of compensatory substitutions are strictly advantageous. Second, the proportion of advantageous to deleterious substitutions has a minimum of 0.5 (for small mutation size), and the proportion of advantageous to deleterious substitutions has a maximum of 0.5 (for very low environmental variability). Third, the magnitude of the coefficient of variation of selection coefficients is near to one. Again, this occurs because these parameters are determined by the evolutionary process and not by *a priori* decisions.

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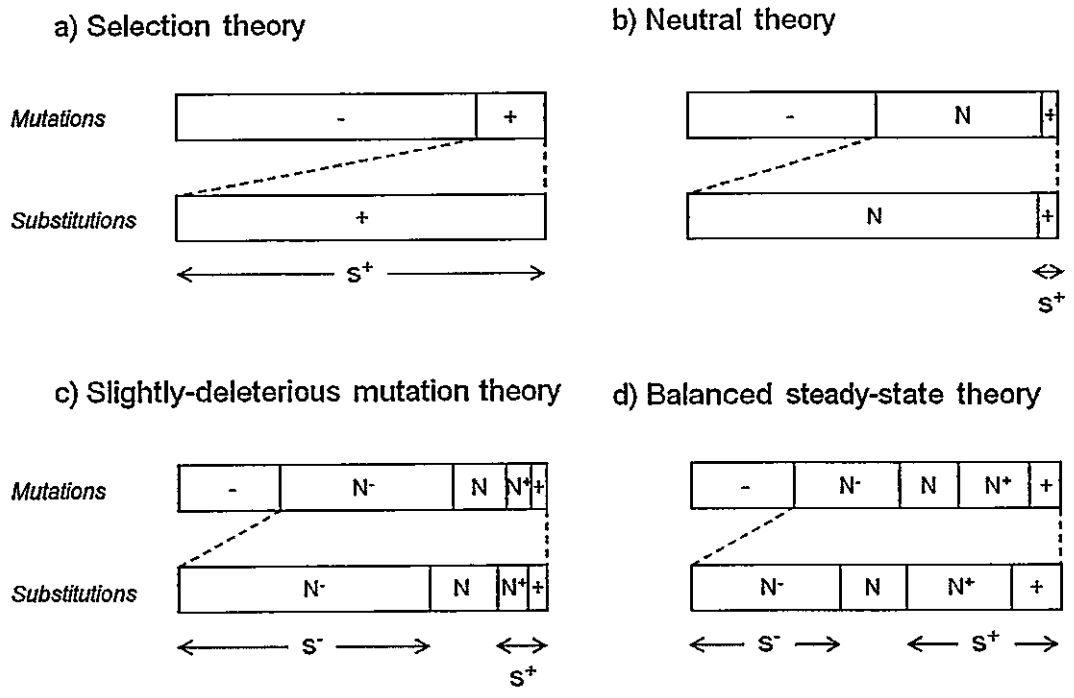


Figure 1. a) The selection (or neo-Darwinian) theory postulates the existence of deleterious (-) and advantageous (+) mutational changes. Deleterious mutations are immediately rejected by negative (or purifying) selection. The 100% of substitutions are a positive selection coefficient $s>0$. b) The neutral theory (Kimura 1968, 1983) postulated the existence of an important fraction of neutral mutations (N) and a very little fraction of advantageous mutations. Neutral mutations are fixed by random drift and constitute the majority of substitutions. A very minority of substitutions have $s>0$. c) The slightly-deleterious mutation theory or nearly-neutral theory (Ohta 1972, 1992, 2002) included mutations between neutral and advantageous (N^+), as well as between neutral and deleterious (N^-). These nearly-neutral mutations are fixed by random drift too, and constitute, with the neutral, the majority of substitutions. The majority of substitutions have $s<0$. d) The dynamic steady-state theory incorporates the nearly-neutral mutations, but also

postulates an important fraction of advantageous (compensatory) mutations fixed after the fixations of slightly deleterious (N^-) mutations. Compensatory mutations are fixed by natural selection and constitute an important fraction of substitutions. The 50% of substitutions have $s>0$ and 50% have $s<0$. (Adapted from Ohta 1992, Bromham & Penny 2003)

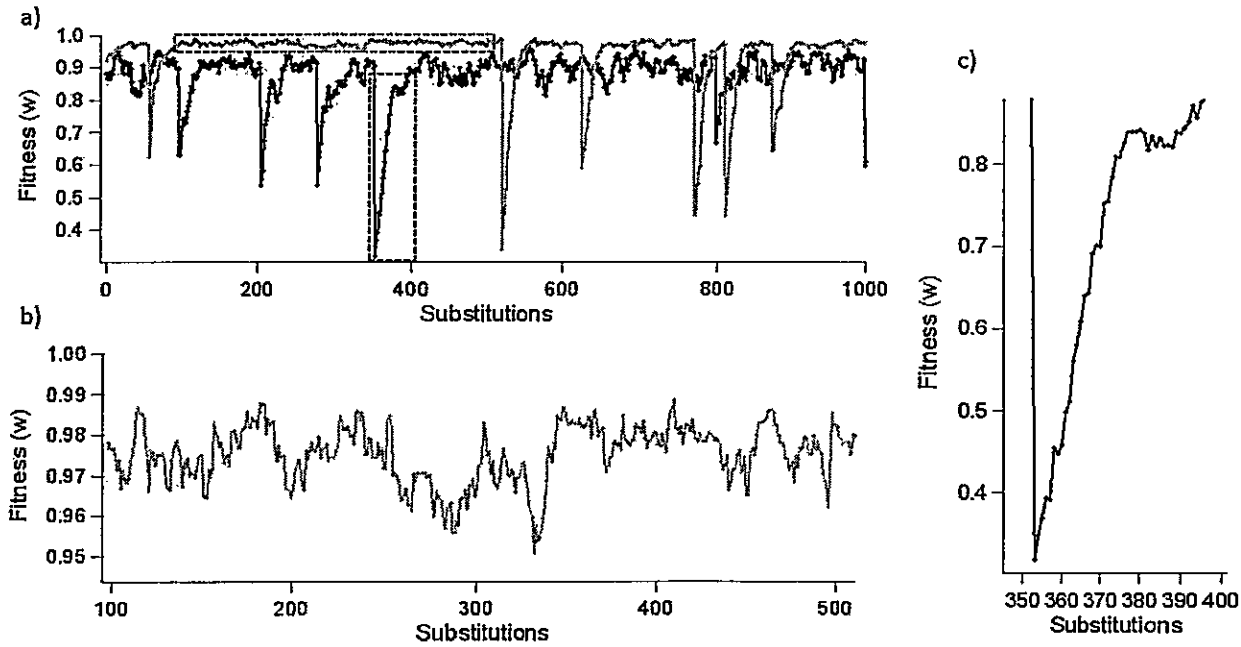


Figure 2. a) Fitness of the evolutionary process for subsequent substitutions with a randomly shifting optimum (variable geometric model). Darker curve corresponds to population size of $N = 50$ ($\tau = 5 \cdot 10^5$), brighter curve corresponds to $N = 200$ ($\tau = 10^5$). b) Zoom of the balanced steady state for $N = 200$ indicated by a dashed horizontal square in a). c) Zoom of the adaptive bout for $N = 50$ by a dashed vertical square in a). Simulation parameters were $n = 20$ dimensions, mean step size $r/2 = 0.3$, shift amplitude $\sigma_a = 0.85$.

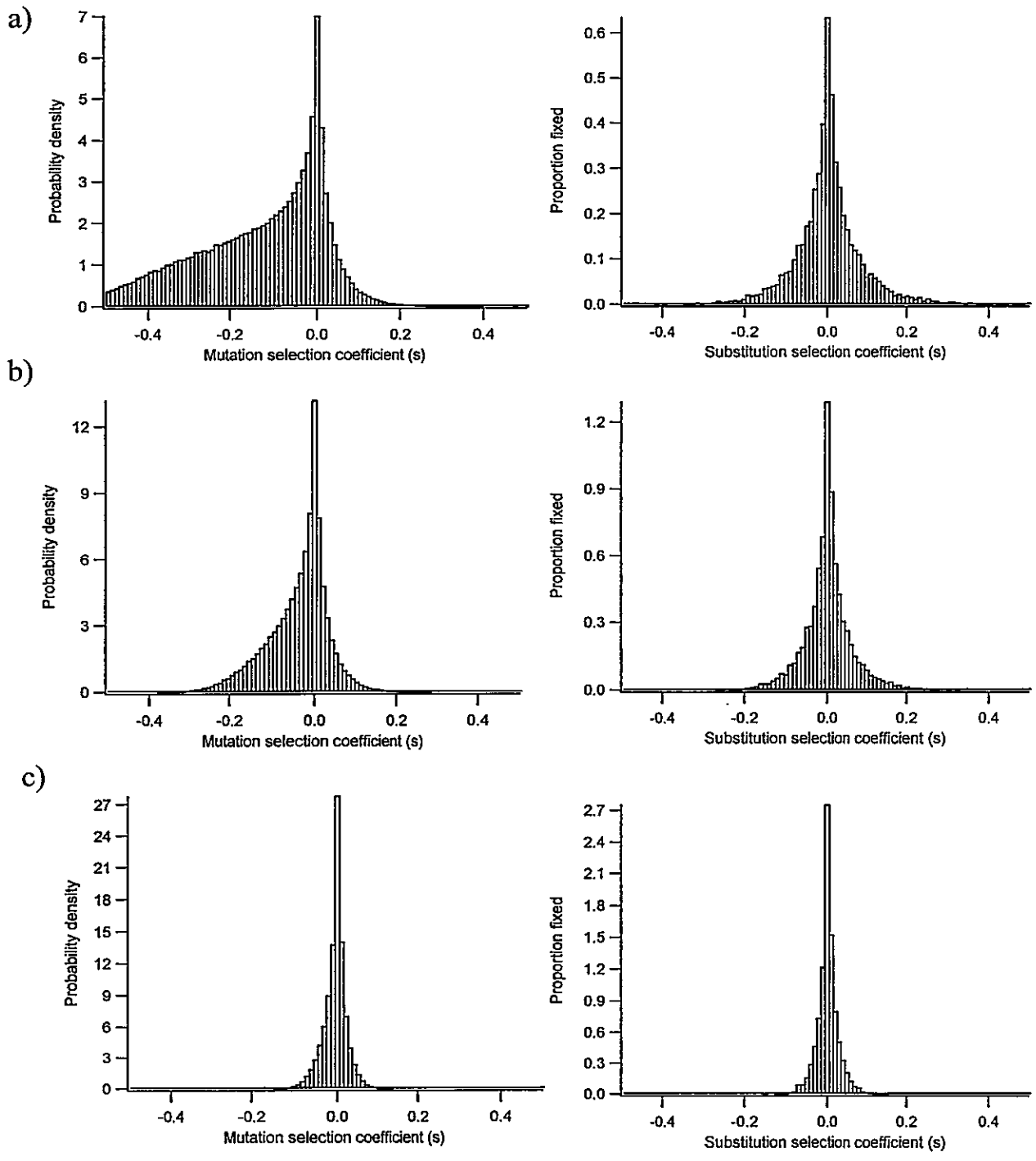
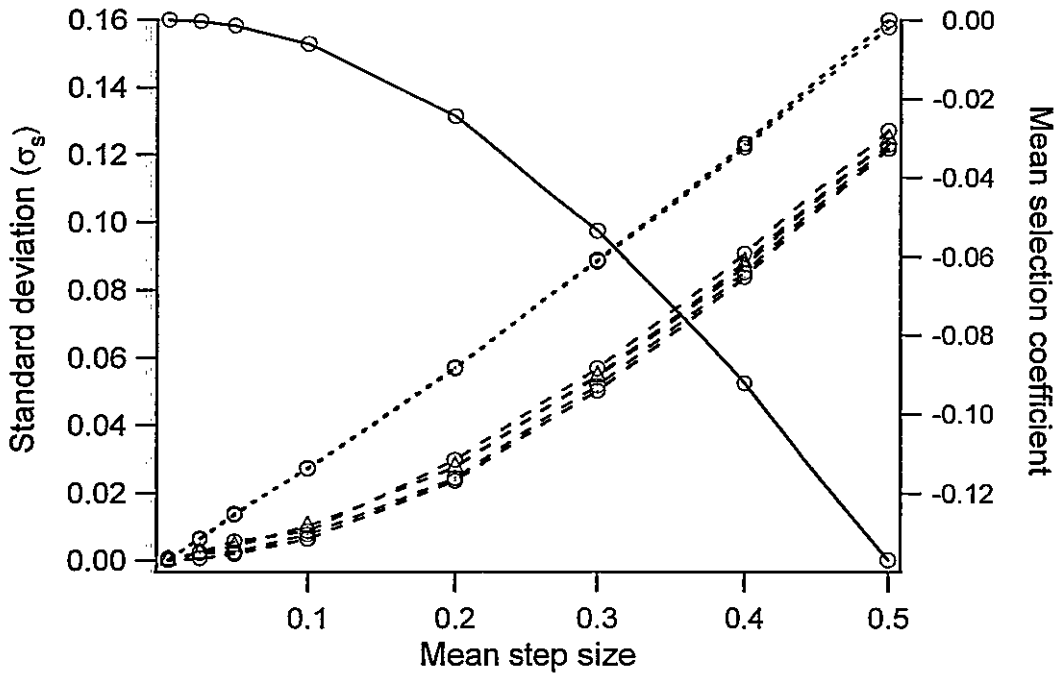

a)
Probability density
Mutation selection coefficient (s)
Proportion fixed
Substitution selection coefficient (s)
b)
Probability density
Mutation selection coefficient (s)
Proportion fixed
Substitution selection coefficient (s)
c)
Probability density
Mutation selection coefficient (s)
Proportion fixed
Substitution selection coefficient (s)

Figure 3. Probability density of selection coefficients of mutations (left) and the proportion fixed (right) in the balanced steady state for different average mutation sizes: a) $r/2=0.5$, b) $r/2=0.25$, c) $r/2=0.01$. General parameters used were $N=10$, $n=5$. Each distribution was constructed for 10^4 substitutions.

a)


0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Standard deviation (σ_s)
0.00
-0.02
-0.04
-0.06
-0.08
-0.10
-0.12
Mean selection coefficient
0.1
0.2
0.3
0.4
0.5
Mean step size

b)

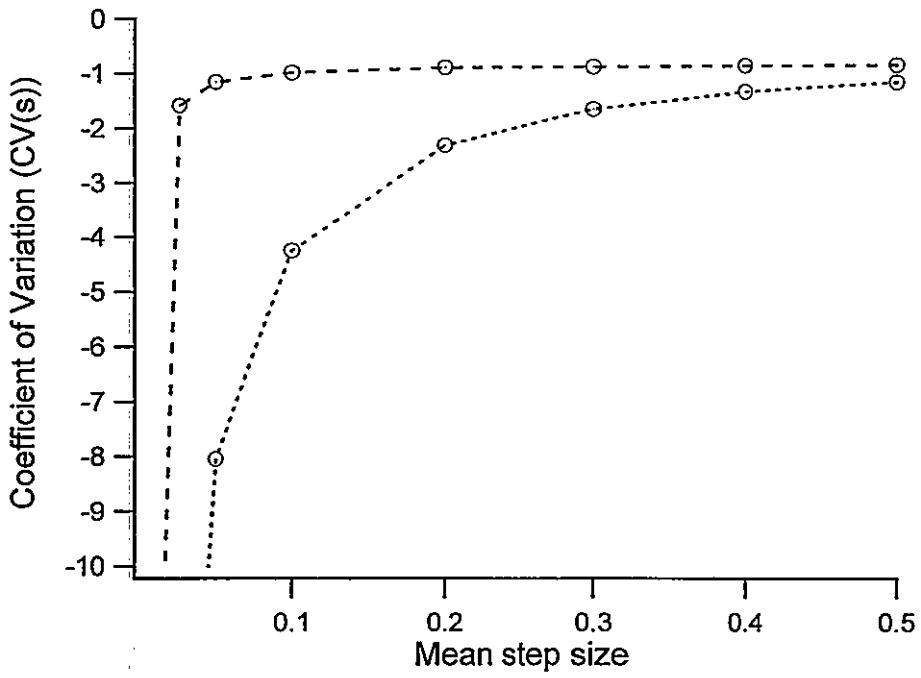

0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
Coefficient of Variation (CV(s))
0.1
0.2
0.3
0.4
0.5
Mean step size

Figure 4. a) Mean selection coefficient (solid line and right scale) and standard deviation of selection coefficients (left scale) for different mean step size ($r/2$). Dotted lines correspond to effective population size $N=10$, and dashed lines correspond to $N=100$ (triangles) and $N=1000$ and $N=10,000$ (circles), which are indistinguishable. Lines are superposed for different dimensions ($n=3$, $n=30$) and different environmental variability and amplitude (fixed and shifting optimum with $\tau=10^4$, $\sigma_a=0.425$). b) Coefficient of variation of selection coefficients for different mean step sizes. $N=1000$ (dashed line), $N=10$ (dotted line).

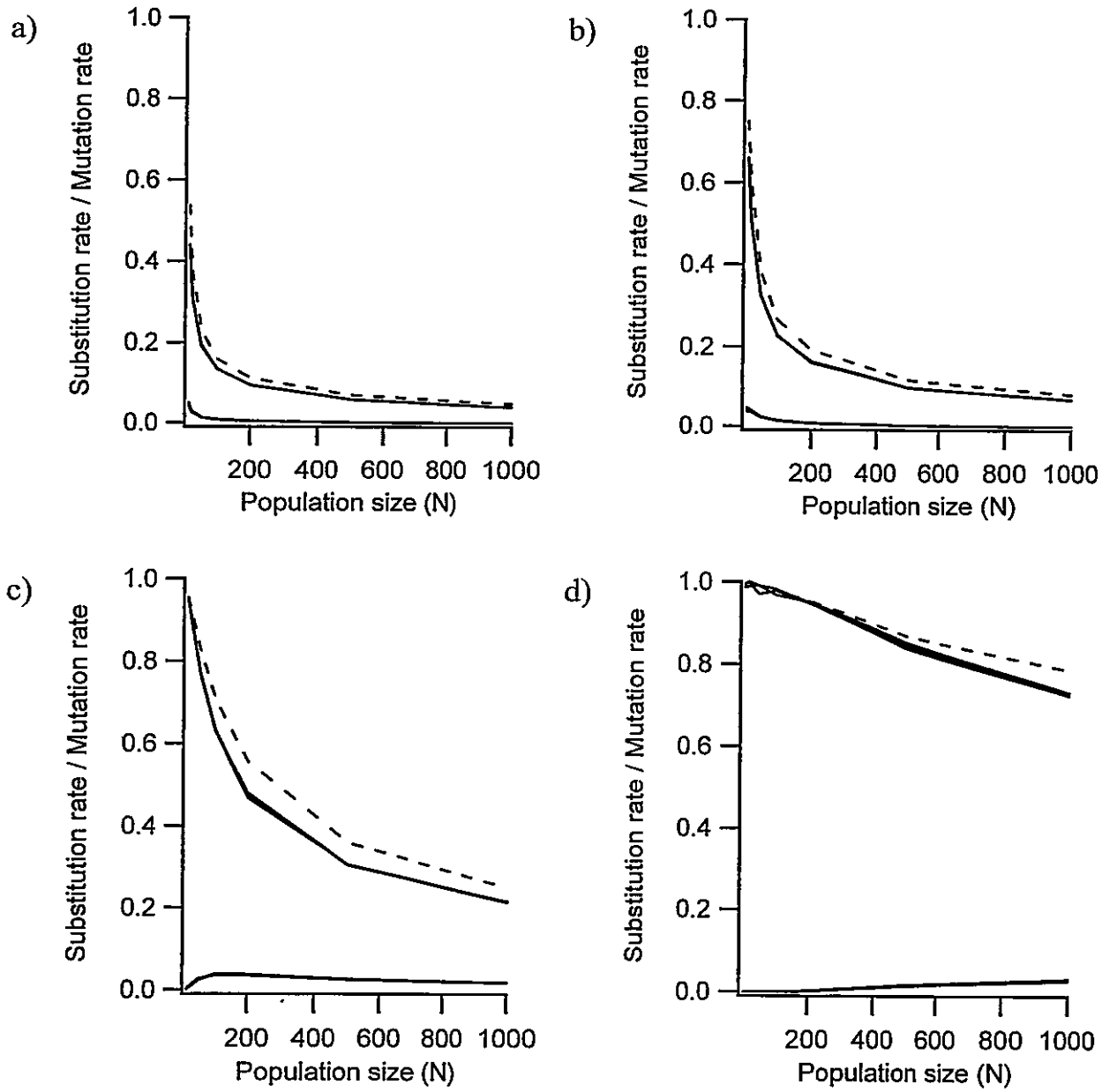


Figure 5. Ratio of substitution rate / mutation rate in relation to population sizes for different size of mutation effects. a) Average size $r/2 = 0.5$ ($\bar{s} \approx -0.13, \sigma_s \approx 0.13$). b) $r/2 = 0.3$ ($\bar{s} \approx -0.055$, $\sigma_s \approx 0.055$), c) $r/2 = 0.1$ ($\bar{s} \approx -0.01$, $\sigma_s \approx 0.13$), d) $r/2 = 0.0025$ ($\bar{s} \approx 0$, $\sigma_s \approx 0$). Dashed lines correspond to total substitution rate. The upper continues lines correspond to neutral substitutions. The lower continuous lines

correspond to strictly advantageous and strictly deleterious ($|Ns|<1$) substitution rate (that are indistinguishable one from the others). All plots were obtained for dimensions $n=2$, $n=10$, $n=30$, but all are perfectly superposed and indistinguishable. Each simulation corresponds to $2\cdot10^4$ substitutions in the steady state.

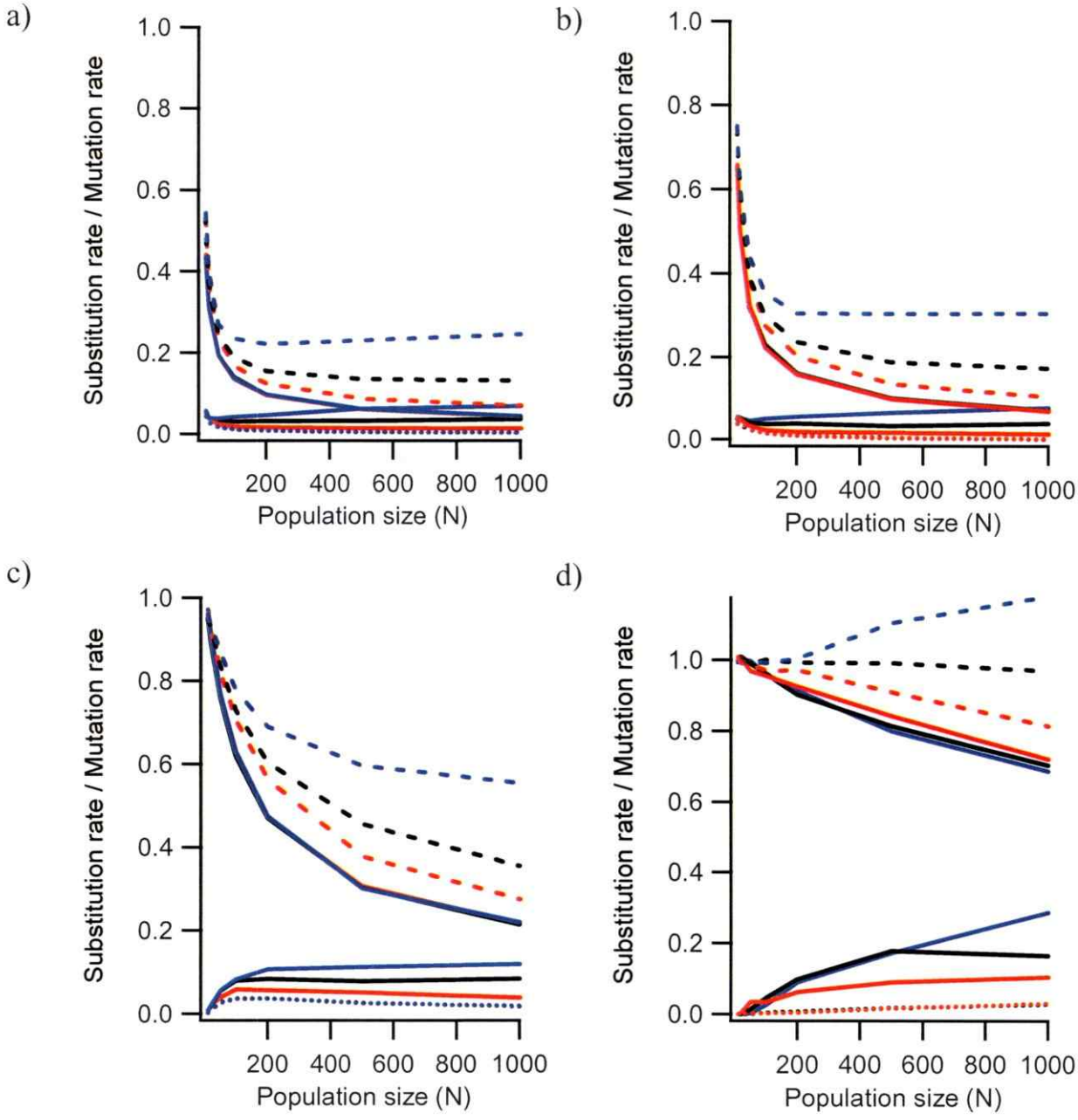


Figure 6. Substitution rate / mutation rate vs. population sizes for different size of mutation effects. a) Average size $r/2 = 0.5$ ($\bar{s} \approx -0.13, \sigma_s \approx 0.13$). b) $r/2 = 0.3$ ($\bar{s} \approx -0.055$, $\sigma_s \approx 0.055$), c) $r/2 = 0.1$ ($\bar{s} \approx -0.01$, $\sigma_s \approx 0.13$), d) $r/2 = 0.0025$ ($\bar{s} \approx 0$, $\sigma_s \approx 0$). Dashed lines correspond to total substitution rate. The upper continues lines correspond to

neutral substitutions. The lower continues lines correspond to strictly advantageous ($s > 1/N$) substitution rate. Dotted lines correspond to strictly deleterious ($s < 1/N$) substitution rate. All plots were obtained for dimensions $n = 2$ (red), $n = 10$ (black), $n = 30$ (blue). Each simulation corresponds to $2 \cdot 10^4$ substitutions. The environmental variability parameters were $\sigma_a = 0.85$, $\tau = 500 \cdot N$. Each curve corresponds to 140,000 substitutions.

CAPÍTULO 2

EL ROL CREATIVO DE LA SELECCIÓN NATURAL

SECCIÓN 1

Tests of adaptation: what do and do not prove

ABSTRACT: It is a general consensus that natural selection can cause and explain the change of frequency of traits in populations. If a trait is propagated and fixed by natural selection, it is called a historical adaptation, and many tests are directed to prove this effect. Nevertheless, beyond this "positive" effect, many biologists hold the claim that natural selection not only can explain the frequency change of already existent traits but also can cause and explain the origin and formation of traits, i.e., natural selection has a "creative" effect able to generate new traits through generations. In this case, these traits are called historical adaptations too. Although there are many tests of adaptation, it is not clear what they do and they do not prove. We review the majority of current tests of adaptation grouping them in three main categories: genetic, phenotypic and phylogenetic, according to the kind of information used. Although the creative role of natural selection has been considered a fundamental thesis of Darwin's theory, there are no tests straightly directed to prove the creative effect of natural selection. However, a number of tests can be complementarily integrated in this task.

KEY WORDS: tests of adaptation, frequency hypothesis, origin hypothesis, creative effect of natural selection, complexity, functional synthesis

INTRODUCTION

The origin of variations and the origin of traits are very different problems. Inheritable variations can be formed from one generation to the next, and it is a well-known fact that the probability of its arising is independent of its adaptive effect (Lenski & Mittler 1993). Nevertheless, the majority of interesting traits for biology are not structures consisting only of a single variation, but its evolutionary acquisition involves at least more than one variation acumulated through many generations. Then, the origin of biological traits can importantly involve the natural selection action, in which case they are called "adaptations". As a matter of fact, the major challenge of the adaptationist research program of evolutionary biology as started by Charles Darwin in the *Origin of Species* is how traits are formed. Indeed, Darwin stressed this challenge in this phrase: "the eye with all its inimitable contrivances for adjusting the focus to different distances, for admitting different amounts of light, and for the correction of spherical and chromatic aberration, could have been formed by natural selection... the difficulty of believing that a perfect and complex eye could be formed by natural selection, though insuperable by our imagination, should not be considered as subversive of the theory [of natural selection]" (Darwin 1872, Ch. 6, p. 144).

The current status of the adaptationist program is based on the modern evolutionary synthesis (Huxley 1942). Nevertheless, the evolutionary synthesis established natural selection as the director of adaptive evolution by eliminating competing explanations (Mayr 1993), but not by providing evidence that natural selection could, or did, account for observed adaptations (Leigh 1999). Since the 60's-70's many types of formal tests of adaptation have been progressively incorporated to the scientific literature overcoming that

shortcoming. Most of those tests incorporate statistical methods for hypothesis testing and they use different approaches and different target null hypotheses. Nevertheless, because the null hypotheses underlay different assumptions and because they use different concepts of adaptation, it is not very clear what tests do and do not prove.

Two meanings of the word "adaptation" are currently being used. Firstly, when a trait or trait variant confers a higher relative fitness in a given environment, it is called an adaptation in a nonhistorical sense (*sensu* Reeve & Sherman 1993), no matter the frequency of the trait or its evolutionary acquisition. Secondly, when the evolutionary acquisition occurs by natural selection the trait is called an adaptation in a historical sense (*sensu* Reeve & Sherman 1993). Nevertheless, within the historical definition of adaptation, we should distinguish between two modes of trait acquisition. Natural selection (NS) explains the acquisition of a trait by a species if NS is the cause of propagation and fixation of the trait. In this sense, natural selection is involved in the explanation of the species' acquisition of an already existent trait but it is not involved in the explanation of the original arising of a previously nonexistent trait.

On the other hand, many authors agree with the claim that natural selection is not reduced to cause the simple propagation of traits or traits variants, but rather NS can be the cause of their existence, i.e., can be the cause of origin, construction, or formation of traits or traits variants (Fisher 1934, Huxley 1942, Simpson 1947, Mayr 1963, Ayala 1970, Dawkins 1986, Gould 2002, Ayala 2007, Avise & Ayala 2007).

Thus, a trait T is called an adaptation in the historical sense, if fulfills one of these two conditions:

1) The spreading and fixation of an already existent individual trait or trait variant T is caused by natural selection. We will call it, the "fixation hypothesis".

2) The formation of a yet non-existent individual trait or trait variant T is caused by natural selection, henceforth the "origin hypothesis".

Usually it is assumed that the formation of a simple trait variant is explained by genetic mutation, and not by natural selection, and thus, natural selection would only explain its propagation and fixation – not its origin (e.g., Endler 1986: 51). In this case, the fixation hypothesis is met but not the origin hypothesis. But, what does it mean that natural selection originates a trait? Simpson (1947) answered this question with the following metaphor (popularized with modifications by Dawkins 1986):

"How natural selection works as a creative process can perhaps best be explained by a very much oversimplified analogy. Suppose that from a pool of all the letters of the alphabet in large, equal abundance you tried to draw simultaneously the letters *c*, *a*, and *t*, in order to achieve a purposeful combination of these into the word "cat". Drawing out three letters at a time and then discarding them if they did not form this useful combination, you obviously would have very little chance of achieving your purpose. You might spend days, weeks, or even years at your task before you finally succeeded... Indeed, you might well never succeed... But now suppose that every time you draw a *c*, an *a* or a *t* in a wrong combination, you are allowed to put theses desirable letters back in the pool and to discard the undesirable letters... Your chances of quickly obtaining the desired result are improved... and by these processes you have "generated a high degree of improbability" –you have made it probable that you will quickly achieve the combination *cat*, which was so improbable at the outset. Moreover, you have created something. You did not create the letters *c*, *a*, and *t*, but you have created the word "*cat*", which did not exist when you started."

Because most biologically relevant traits are complex, the cause or explanation of their origin does not consist simply in a single genetic change. When the trait formation involves a series of structural transformations, the explanation of its origin is hence the explanation of the occurrence of such series. If the series of inter-generational changes that originate a trait is not possible without a specific factor, and if this factor considerably increases its probability, this factor has a causal role in its origination. This factor is probabilistically indispensable. Thus, the causal role of natural selection in the origin of traits is a probabilistic causality (see Box 1). In this sense, the power of natural selection to generate complex adaptations consists of its capability to make more probable coherent combinations of events that result in complex structures that seem impossible to produce by the random accumulation of changes. This is a creative process (Fisher 1934, Huxley 1942, Simpson 1947, Mayr 1963, Ayala 1970, Dawkins 1986, Gould 2002).

In genetic terms, any genetic substitution fixed by positive selection has, at the same time, the "destructive" effect of losing the previous monomorphic or polymorphic "wild-types" at a locus. Furthermore, the phenomenon called hitchhiking effect of selective sweeps (Gillespie 2000) can magnify the destructive effect by carrying-over neighboring genomic sites. Nevertheless, this lost of variability may be part of a creative process. Positive selection is necessary for the creative effect of natural selection, but creative selection also requires that the fixation of changes be accumulative and directional (i.e., functionally coherent). In this way, to prove isolated events of positive selection is not sufficient to prove the origin hypothesis. In other words, positive selection could explain why traits have the frequencies they do, but not why individual organisms have the traits they do.

Each one of these two questions refers to the fixation and origin hypotheses, respectively. But, do the current tests of adaptation prove these fundamental hypotheses? Available tests address several hypotheses and sub-hypotheses via particular approaches depending on theoretical assumptions, statistical methods, and available data, among others. Up to now there is no systematic analysis of what tests of adaptation do and do not prove. Here, we try to fill this gap.

As we will show in the next pages, the majority of tests of adaptation do not tackle directly the origin question, but rather the propagation question. We first review a broad (although not exhaustive) spectrum of methods developed to assess adaptation in natural systems, and analyze the target of those tests and their explanatory scope. Second, we aim to integrate the different approaches seeking a unified explanatory framework, and discuss some of the causes of the bias towards the propagation question.

GENETIC TESTS OF NATURAL ADAPTATIONS

Comparatively with the phenotypic and phylogenetic tests, genetic tests of adaptations have a short history. They began only when the neutral theory of molecular evolution (Kimura 1968a, 1968b, 1983) allowed generating well established null models that make predictions as the null hypotheses for molecular evolutionary data analysis (Nielsen 2005). The different tests are based in genetic information but are centered in different target of null hypotheses (Table 1). Tests are based on the fact that natural selection may affect levels of variability, linkage disequilibrium, haplotype structure, allelic distribution in each nucleotide site and differences between classes of mutations present as polymorphisms or

substitutions (Nielsen 2005). All of these tests interpret adaptation as an explanation of the deviation from the null neutral expectation and one of its main interests is detecting genes, or genomic regions, that have been targeted by natural selection. Briefly we describe several genetic tests and its goals. In the discussion we describe some current controversies about what they do and do not prove. We will distinguish between the tests that discriminate the different types of mutations (synonymous vs. nonsynonymous), henceforth the "Type of mutation approach", and the tests that do not discriminate between types of mutations, the "Indiscriminate mutation approach".

Indiscriminate mutation approach

The majority of tests belong to this class. A category is based on the expectation about the differences in allele frequency distribution of allelic samples in nucleotide sites (frequency spectrum) and/or level of variability of genetic changes. In a neutral model, the expected value of the number of mutations that exist in a frequency x_i is proportional to $1/i$ (where $x_i = i/n$ for $i = 1, 2, \ldots, n-1$, in a sample of size n). The predomination of different evolutionary forces will have different effects on the frequency spectrum. For example, negative (purifying) selection will increase the fraction of mutations at low frequencies in the sample. Conversely, positive selection will tend to increase the frequency in a sample of mutations segregating at high frequencies (Nielsen 2005). A selective sweep (the process that reduces variation in linked neutral sites of advantageous mutations that increase in frequency by positive selection) has roughly the same effect of purifying selection.

The tests that are probably the most commonly applied neutrality tests to date (including molecular ecology studies) are the Tajima's D-test (Tajima 1989) and its derivates, where the average number of nucleotide differences between pairs of sequences is compared with the total number of segregating sites (SNPs). If the difference between these two measures of variability is larger than what is expected on the standard neutral model, this model is rejected. The H-test or Fu-F test (Fu & Li 1993) is an extension of the Tajima's D-test to take information regarding the polarity of the information into account by the use of an evolutionary outgroup (e.g., a chimpanzee in the analysis of human genetic variation), and more refinements were introduced by Fu (1996, 1997). Fay & Wu (2000) developed a new statistic (H) that is sensitive to an excess of new marker alleles at high frequency, which is consistent with hitchhiking but not background selection (the process that lows the probability of persistence of a mutation neighboring deleterious alleles). This new statistic test can be used in combination with other statistic tests (such as Tajima's D) to reveal more about the pattern of selection. A significantly negative D is consistent with selective sweep, but it is consistent with many other processes as well. If H is significant also, then background selection or a recent population expansion can be ruled out. Conversely, if D is significant but not H, other explanations besides selective sweep gain credibility for the reduced level of genetic variation (Otto 2000).

Other tests utilize genetic linkage disequilibrium (LD) information (Emelianov et al. 2004; Scotti-Saintagne et al. 2004), i.e., the correlation among alleles from different loci, or available genome sequence (Harr et al. 2002; Schlötterer 2003) to identify specific chromosomal regions, that are affected by positive selection. Selective sweeps increase levels of LD in a transient phase, although this phase may be relatively short (Przeworski

2002). This genomic scans have been the subject of great interest because they have the potential to inform us about very recent adaptive evolution, and this is of particular interest for understanding the evolution of our own species, however, they can only tell us the general location of an substitution fixed by natural selection, they do not tell us which mutation or even which gene was responsible for the event (Eyre-Walker 2006).

A major interest of tests based on differences in allele frequency distribution and level of variability is that may make possible to pinpoint the location of a selective sweep. Nevertheless, it has been questioned the capacity of these tests to make robust inferences of selection as they depend on strong assumptions regarding population demographics, such as no population structure and constant population size (Nielsen 2001). Because the population genetic neutrality tests are affected by the population's demographic history, their results have often been contentious and have not led to firm conclusions regarding the action of selection. Generally the effects of selection processes are hardly distinguishable from the effects of demographic processes.

Positive selection could be distinguished from demographic effects because selection produces a skew in local molecular signatures while demography produces a genome-wide skew. Some methods sometimes reveal evidence for positive selection in individual genes by multilocus comparisons and detecting outlier loci. A category of tests compare the level of divergence between two or more samples to detect outlier loci that exhibit higher (or lower) than expected intergeneration/population/species differentiation (commonly measured as F_{ST} or analogous statistic). One of the first neutrality tests proposed, Lewontin-Krakauer test (Lewontin & Krakauer 1973) uses the variance values of Fst (Wright's fixation index) among loci to identify those loci that deviated more than

expected. This approach has been developed due to the availability of large-scale genomic data (Beaumont & Nichols 1996, Suzuki & Gojobori 1999, Schlotterer 2002b, Akey et al. 2002, Kayser et al. 2003, Beaumont & Balding 2004).

Another test based on the outlier loci detection is the Hudson, Kreitman & Aguade (1987) or HKA test, which compares variation with and between species (polymorphisms/substitutions) for multiple genes. Selection may in many cases increase the degree of differentiation among populations. For example, a selective sweep tends to drastically reduce variation within a population, but will not lead to a reduction in differences between species. Neutrality is rejected if the ratio varies more among genes than expected on a neutral model. Other tests are based on recent theory showing that a selective sweep can have a strong impact on the level of population subdivision, particularly when the sweep has not yet spread to all populations within a species (Charlesworth et al. 1997, Slatkin & Wiehe 1998, Majewski & Cohan 1999). When a locus shows significantly high levels of genetic population differentiation, compared with other loci, this may then be interpreted as evidence for positive selection (Nielsen 2005). Differential adaptation, or artificial selection on traits, can lead to large between-population allele frequency differences at the loci that control the traits involved. The degree to which tests for outlier loci are robust to the underlying demographic assumptions is controversial and has not been fully explored (Nielsen 2005). Finally, the ln RV and ln RH tests utilize highly variable microsatellite loci to compare genetic diversity between two samples by calculating the variance (V) in allele size, and the expected heterozygosity (H), respectively (Shlötterer 2002a). Loci that have been affected by recent strong directional selection are expected to lie in the tails of empirical genome-wide distribution of ln RV and ln RH

(Vasemagi & Primmer 2005). One of the critical prerequisite of both ln RV and ln RH test is the assumption of constant within-locus mutation rate across populations. Given that there is strong correlation between microsatellite repeat number and mutation rate (Neff & Gross 2001) such an expectation is probably unrealistic and different allele-specific mutation rates can potentially have a significant bias to the outcome ln RV and ln RH test (Vasemagi & Primmer 2005). Overall, the major weakness of the Indiscriminate mutation approach is that cannot provide robust inferences of selection as they strongly depend on demographic model (Nielsen 2001, 2005). In contrast, the Type of mutation approach seems to be more robust to demographic factors, which has promoted its use (Nielsen 2005, Eyre-Walker 2006).

Type of mutation approach

These tests usually are based on the differences between classes of mutations within a locus. The tests called d_N/d_S or K_a/K_s estimate the average nonsynonymous (d_N or K_a) and synonymous (d_S or K_s) substitution ratio between two sequences and has been traditionally used to infer whether particular genes have been mainly under negative (purifying) selection ($d_N<d_S$), completely neutral evolution ($d_N=d_S$) or positive selection ($d_N>d_S$) (Kimura 1977, Nei & Gojobori 1986, Li 1993, Ina 1995, Hurst 2002). These tests are considered very robust to detect positive selection (Kimura 1977, Fay & Wu 2001) and usually result in the evidence of wide-spread purifying selection for the most of proteins (i.e. $d_N/d_S<<1$) (Hurst 2002). Thus, it is a very conservative tool, because if most mutations will disrupt function, the amount of positive selection needed to elevate the d_N/d_S above one

is enormous. Rapidly evolving genes have been identified by this criterion. For example it has been demonstrated the presence of positive selection on HIV sequences (Nielsen & Yang 1998) and on the human major histocompatibility locus (Hughes & Nei 1988). Nevertheless, by its conservative nature, the criterion is verified in few instances (often when immune systems genes co-evolve with parasites, Nielsen et al. 2005, Hughes 2007). In order to overcome this problem, methods have been devised that takes variation in the d_N/d_S ratio into account (Nielsen & Yang 1998, Yang & Nielsen 2000). The use of these "codon-based" methods increased the number of loci where it has been detected the presence of positive selection (Stahl & Bishop 2000, Yang & Bielawski 2000, Ford 2002), which has led to some skepticism toward this methodology (Suzuki & Nei 2002, 2004). Statistical methods allow making inferences site-specific regarding positive selection, which enable to determine if specific sites have been targeted by positive (or negative) selection (Fitch et al. 1997, Nielsen & Yang 1998, Suzuki & Gojobori 1999, Zhou et al. 2008, for criticisms to this method see Hughes 2007, 2008, Hughes & Friedman 2008).

In the McDonald-Kreitman (MK) type test (McDonald & Kreitman 1991), the d_N/d_S ratio is compared with the average nonsynonymous and synonymous polymorphism ratio (P_N/P_S) within species. If all mutations are either strongly deleterious or neutral then is expected that $d_N/d_S = P_N/P_S$, but if $d_N/d_S > P_N/P_S$ is understood as some of the nonsynonymous substitutions have been fixed by positive evolution because, on average, adaptive mutations contribute relatively more to substitution than to polymorphism, when compared with neutral mutation (Eyre-Walker 2006). The MK and particularly the d_N/d_S test are robust to demographic assumptions (Nielsen 2001, 2005, but see Hughes 2007) by which it has increased notably its use (Eyre-Walker 2006). The reason is that the sites in

which synonymous and nonsynonymous mutations occur are interspersed among each other and thus they are similarly affected by demography and genetic drift. Unfortunately, given that both nonsynonymous and synonymous mutations, linked to the beneficial mutation, will be similarly affected by the selective sweep, the Type of mutation approach may not be very suitable for detecting recent selective sweeps. Also, the Type of mutation approach cannot distinguish between past and present selection and leads to a significant loss of information because it reduce the information in the data simply to the number of nonsynonymous mutations and synonymous mutations (Nielsen 2005, see Hughes 2007 for other technical criticism to this approach).

In summary, in the genetic tests of natural adaptations, positive selection can be assessed directly on the target of selection or indirectly on linked genomic regions (selective sweeps). In the indirect approach, selection is detected on the basis of a skew in the frequency distribution or variability of neutral variation linked to a site that has been under selection. Positive selection and selective sweeps have different molecular signatures. Positive selection may increase or decrease intraspecific variability, increase interspecific variability, increase the ratio of interspecific to intraspecific variability and increase the proportion of high frequency variants in the frequency spectrum. On the other hand, selective sweeps decrease intraspecific variability, no affect the mean but affect the variance of interspecific variability, increase the ratio of interspecific to intraspecific variability, and mostly increase the proportion of low frequency variants in the frequency spectrum (Nielsen 2005).

As is apparent, both the Indiscriminate mutation and Type mutation approaches are no usually linked to phenotypic information (see Discussion for the exceptions) and the

majority search evidence of the fixation hypothesis for single substitutions or single genes. Thus, difficulty they could undertake the origin hypothesis by itself. Nevertheless, if we define a protein as a complex trait that could be formed by natural selection, then d_N/ds and MK tests could be understood as testing the creative role of natural selection in the protein adaptive formation, being the protein itself an historical adaptation formed by the continuous adaptive sequence of changes that improve the performance of the protein. Nevertheless, it have been questioned that thing be proved by this tests because it is possible that the positive selection could only work to maintain the *status quo* and not to continue adaptation of the protein (Hartl & Taubes 1996, Kondrashov et al. 2002, DePristo et al. 2006, Pal et al. 2006, Camps et al. 2007) (see Discussion).

PHENOTYPIC TESTS OF NATURAL ADAPTATIONS

The analysis of the phenotypic variability of traits due to natural selection is among the oldest approaches used to assess adaptive hypotheses. We here have separated intra-specific and inter-specific tests (see below Phylogenetic tests of natural adaptations), given that they focus at different levels of variability. Two broad approaches can be distinguished for assessing natural adaptations at the phenotypic level, here broadly named, (i) the correlational method, and (ii) the experimental method. Each of these two approaches comprises a large family of tests, with different traditions and methods, and they encompasses from spatial and/or temporal comparisons within and between populations, to descriptions of population variability and its relationship with fitness, to tracking controlled reproduction in target populations, to optimality and evolutionary stability models and

testing to environmental and phenotypic manipulations, and to artificial selection, among others. In this review we have focused only on natural adaptations, and as it will be shown, all tests focus on the "fixation hypothesis" of adaptation.

Correlational approach

The basic aim of this approach is to detect if there is a correlation between the character (or trait) and an environmental variable (e.g., assessing geographic variation, or temporal (trans-generational) variation) and/or between a trait and fitness or a surrogate variable related to survival and/or reproduction (selection assessment). A comprehensive correlational study should include assessments of the three conditions that define natural selection at the individual level according to Endler (1986), that is: (i) variation, i.e., variation among individuals in some attribute or trait, (ii) fitness differences, i.e., a consistent relationship between that trait and mating ability, fertilizing ability, fecundity, and/or survivorship, and (iii) inheritance, i.e., a consistent relationship, for that trait, between parents and their offspring, which is at least partially independent of common environment. If these 3 conditions are accomplished, then the process (*sensu* Endler 1986) of natural selection occurs.

One of the most traditional correlational approaches to assess the adaptive value of traits has been the analysis of geographic variation. If natural selection occurs, then geographic variation in selective actors give rise to parallel geographic variation in traits when the effects of genotype-environment factors have been controlled (Endler 1986, see e.g., Thompson 1991). More comprehensively, the assessment of variation comprises the

analysis of trait distribution within a population, while fitness differences are studied by analyzing survival and/or reproductive output (Lande & Arnold 1983, Kingsolver et al. 2001, Halama & Reznick 2001), although most frequently surrogate variables are measured instead (such as foraging ability, physiological condition, among others) (e.g., Ritchie 1990, see also Halama & Reznick 2001). Given a selection differential in a population trait distribution, a response to selection can be assessed via analyzing the inheritance of traits between generations (Fairbairn & Reeve 2001). Inheritance is normally studied by assessing heritability, that is, the fraction of observed phenotypic variance for which differences in heredity are responsible (Feldman 1992, Lynch & Walsh 1998). If heritability is nil, no response to selection can occur.

Around 25-30 years ago, a number of new methods were introduced with the aim of assessing the strength of selection on multiple quantitative traits (Lande 1979, Lande & Arnold 1983, Arnold & Wade 1984, see also Kingsolver et al. 2001). These methods allowed distinguishing the direct and indirect components of selection on a set of correlated characters, hence assessing selections gradients (i.e., the relationship of relative fitness to the variation in a quantitative trait), which in turn, were relevant to quantitative genetic models for the evolution of multiple and correlated traits (Lande 1983; see also Kingsolver et al. 2001).

Experimental approach

More than a single specific method, this approach encompasses several different theoretical and manipulative methods that share the common interest of finding a causal relationship

between phenotypic structures or traits and fitness benefits. Some of these methods rely on theoretical developments such as optimality and game theory modeling, followed by experimentation o field assessments (e.g., Boyce & Perrins 1987, Sinervo 1996), while others use diverse experimental (manipulative) methods to assess the adaptive value of phenotypes (e.g., Andersson 1982, Basolo 1990, Moller 1992, Vásquez & Kacelnik 2000, see Sinervo & Basolo 1996 for a review). A particular branch of methods, artificial selection, use experimental populations under controlled conditions to carry out selective breeding, for example, choosing certain traits across generation, in order to increase (or decrease) the value of the chosen trait (e.g., fur color in a mammal species). In this review we have focused on natural variation of natural populations, and hence, artificial selection has been left out (for artificial selection literature, see Mackay & Falconer 1996, Rose et al. 1996, Garland & Rose 2009).

One traditional method used to assess the adaptive value of traits has been optimality theory (Parker & Maynard Smith 1990). This method basically relies on developing a hypothesis about the fitness value of a trait of an organism that it thought to have evolved in response to an identifiable form of natural selection (see Reeve & Sherman 1993, Orzack & Sober 1994, Reznick & Travis 2001). In this sense, there is a cause-and-effect relationship (see Grafen 1988, Orzack & Sober 1994). First, the method involves the construction of a theoretical model about adaptation, by asking an explicit question. For instance, why is the sex ratio of most species the unity? (following Parker & Maynard Smith 1990). A second stage encompasses the definition of alternative strategies, i.e., a set of phenotypic variants. For the sex ratio example, it would involve all the ratio points from producing exclusively one sex to producing exclusively the other sex. The strategy set

specifies the plausible alternatives that evolution is thought to produce, and the model includes an assumption about what alternative phenotype generates the highest (maximum) fitness, and an optimization criterion is developed often using an indirect or surrogate measure of fitness (e.g., measures of lifetime surviving offsprings, survival probability, energy efficiency, foraging success, among others; see Parker & Maynard Smith 1990). The model includes assumptions about the fitness consequences of the different phenotypic variants. Fitness functions are often generated empirically, commonly by experimentation (e.g., Lemon 1991), but sometimes using natural variation (e.g., Boyce & Perrins 1987). Once payoffs to the phenotypic variants have been stated, the optimal solution (or solutions) is deduced by an appropriate analytic technique such as direct optimization (see e.g., Stephens & Krebs 1986, Houston & McNamara 1999, Clark & Mangel 2000) or stability analyses using game theory (see e.g., Maynard Smith 1982, Dugatkin 1998). The final step is to test the predictions (quantitatively or quantitatively) against the observation; if they fit, the model may really reflect the force or forces that have shaped the adaptation (Parker & Maynard Smith 1990).

The phenomena being studied may encompass two types of selection that require two different theoretical tools: (i) if selection is frequency-independent, then simple optimization is used, and the optimal phenotypic variant is found (see Ritchie 1990, Boyce & Perrins 1986, Pettifor et al. 2001, Vásquez & Kacelnik 2000, for examples; see also Stephens & Krebs 1986, Houston & McNamara 1999, Clark & Mangel 2000, for reviews), (ii) if selection is frequency-dependent, then stability tools using mathematical techniques derived from game theory are used (see Gross & Charnov 1980, Herre 1987, Sinervo &

Lively 1996, Ross-Gillespie et al. 2007, for examples; see also Maynard Smith 1982, Parker & Maynard Smith 1990, Dugatkin 1998, for reviews).

Another experimental approaches being used to assess the adaptive significance of traits include manipulation of the phenotype and manipulation of the environment (see Sinervo & Basolo 1996 for a review). The aim of these two methods is to assess the influence of a given trait (or trait value) to fitness or to a component of fitness. Thus, manipulations are aimed to assess the adaptive nature of trait variation. Experimental manipulations provide additional insight into the adaptive nature of traits because they make possible to separate the effects of natural selection from stochastic effects (Sinervo & Basolo 1996). Phenotypic manipulations may comprise the modification of traits, such as egg size (Sinervo et al. 1992), brood or clutch size (Gustafsson & Sutherland 1988, Pettifor et al. 1988), hormone level (Ketterson et al. 1992, Mills et al. 2009), immune response (Moreno et al. 1999), external morphology including feather length in birds (Andersson 1982, Moller 1992), and caudal fin length in fishes (Basolo 1990), and behavioral traits such as call features in frogs (Ryan et al. 1990), among others. For example, by studying the survival of a large number of control and experimentally manipulated offspring to maturity, Sinervo et al. (1992) tested whether the observed seasonal changes in egg size were adaptive; females typically produce larger offspring in later clutches, accordingly with measurements of natural selection on offspring size (Sinervo et al. 1992, see also Sinervo & Basolo 1996).

On the other hand, environmental manipulations may comprise the modification of food availability (e.g., Hirshfield 1980), predation level (e.g., Resnick et al. 1990), competition level (e.g., Schluter 1994), parasites (e.g., Zbinden et al. 2008), and social

environment (e.g., Vásquez & Kacelnik 2000), among others. For example, Hirshfield (1980) manipulated food availability and temperature in rice paddy fish and found correlated effects of feeding success on fitness related variables such as reproductive effort and survival. A similar approach used Lemon (1991), studying assumptions of optimal foraging theory with zebra finches, *Taenyiopygia guttata*; he assigned net rates of energy gain via foraging to randomly chosen individuals for their entire lifetimes and measured fitness directly, and showed that fitness measured as population growth rate was positively correlated with the net rate of energy gain via foraging; hence supporting the assumption that more efficient foraging is adaptive.

PHYLOGENETIC TESTS OF NATURAL ADAPTATIONS

Comprehending the evolution of structurally complex phenotypic features, such as the avian wing or the vertebrate eye or explaining amazing coincidences as aposematism and gregariousness among taxa has been a difficult problem for evolutionary biologists. By examining the nested pattern of appearance of features in a phylogenetic hierarchy, it is possible to reconstruct how a complex structure or biological patterns has evolved through time (Losos et al. 1994). Over the past two decades, the theory and methods of phylogenetic reconstruction have improved dramatically and it has become widely accepted that phylogenies need to be incorporated into the analyses of interspecific data (Losos et al. 1994; Sheldon et al. 1997; Martins 2000). Accordingly, numerical studies using the comparative method - comparison of two phenotypes across a range of species or higher taxa, or a comparison of one phenotype along an environmental variable – have increased,

including the use of phylogenies to interpret historical patterns in ecology, behavior, and morphology (Lauder et al. 1993; Miles et al. 1993; Losos et al. 1994; Martins 1996; Ricklefs 1996; Sheldon et al. 1997; Nunn et al. 2001; Freckleton et al. 2002; Grandcolas et al. 2003; Garland et al. 2005). The use of phylogeny makes possible determine whether a character originally spread in ancestral populations through natural selection for its current function (e.g., Greene 1986; Coddington 1988; Baum et al. 1991), assess whether a character's presence in an extant taxon results from a "phylogenetic constraint" on adaptive evolution (e.g., McLennan et al. 1988; Brooks et al. 1991; Sheldon et al. 1997), and assess whether the evolution of one character was facilitated by the presence of another by determining whether these characters arose in a predicted historical sequence (Sillen-Tullberg 1988; Donoghue 1989; Carpenter 1991; Sillen-Tullberg et al. 1993). Two principal approaches to analyzing character evolution in a phylogenetic context have found broad application in the studies of adaptation (Coddington 1994), (1) the homology approach (also called the "functional" or "homoplasy" approach) and (2) the convergence approach (also called the "cladistic" approach). Here, we analyzed them and also include a different approach based in general phylogenetics trends, which also are utilized in the research on adaptation.

Homology Approach

This approach is characterized by a focus on a particular character in a particular lineage and the attempt to test hypotheses of adaptation using phylogenetic and functional information (Greene 1986; Baum et al. 1991; Coddington 1994). The homology approach

emphasizes the analysis of evolutionary novelties (apomorphies); for example, the origin of the orb as an architectural pattern in spider web (Coddington 1986a; Coddington 1986b; Coddington et al. 1991), the evolution of hypsodonty in equids (Stromberg 2006), leaf retention in the common oak and the beech (Wanntorp 1983; Wanntorp et al. 1990) or morphological features of a strict egg-eating African snake (Gartner et al. 2008). In all mentioned examples the cause of an observed pattern is unique and therefore has its particular historical explanation (i.e., logical individuals; see Ghiselin 1974 and Coddington 1994), even if the trait evolves more than once on the cladogram.

Hence, it is not necessary statistics, where repetitive events are required and thus the hypothesis of adaptation should be corroborated by testing multiple independent deductions against facts (Coddington 1994). Each hypothesis must be rich detailed to offer many points where correspondence to fact can be tested (Coddington 1994; Stromberg 2006). This approach assumes that selection is responsible for the origin and the maintenance of the adaptation and that the nature of the selection is the same throughout (Coddington 1988; Baum et al. 1991; Frumhoff et al. 1994; Grandcolas et al. 2003; Kluge 2005).

In general, in order to test if a trait constitutes an adaptation, it is required to demonstrate that it is an apomorphy, which derived function has some average effect on fitness, given the environment. We show two methods utilized to test adaptation through the homology approach:

1.- *The origin of a novel trait t coincides with the origin of the novel environment e.*

Coddington (1988) proposed that one could test the hypothesis of adaptive origin for a trait of interest simply by mapping the character states of sister taxa onto an independently

derived cladogram. In this scenario adaptation is evident when a trait change occurs at the same location within a phylogeny as the environmental change (Coddington 1988; Miles et al. 1993). If no performance based test of the adaptive hypothesis can be developed, but the cladogram, character polarities and predicted scopes and sequences of events are sound, then at least the plausibility of the adaptive hypothesis has been established. Such assessments could be considered a minimum requirement for an adaptive hypothesis.

2.- *Trait t evolves subsequent to the environmental change e.*

Baum and Larson (1991) suggested that adaptation is evident only when the environmental change precedes the trait change. To evaluate this assumption they proposed to use the notion of selective regime, the aggregation of organismal and environmental factors that combine to determine the expected action of natural selection on actual and potential character variation (e.g., pollination mechanism). Therefore, to constitute an adaptation, *t* should have evolved in a lineage whose selective regime was such that enhanced performance of that biological role was favored (for an example, Baum et al. 2001). In the case that *t* had evolved before of such selective regime it is suggested an exaptation (Gould et al. 1982) and if evolved on the same branch of the selective regime the result is equivocal; the adaptive hypothesis is neither supported nor rejected.

Convergent approach

Contrarily to homology approach the convergence approach (Coddington 1990; Coddington 1994) forgoes detailed study of particular cases to reach for statistically significant correlations between classes (Ghiselin 1974) of non-homologous events that

results to one common cause, natural selection (Coddington 1994; Martins 2000; Garland et al. 2005). The convergence approach discards homology and views homoplasy as the interesting and evidentially powerful source of information and as an evidence of adaptation (Miles et al. 1993). It considers the phylogenetic information to establish evolutionary patterns unbounded by the particulars of history (Felsenstein 1985; Harvey et al. 1991c). The rationale to doing this is based in that interespecific data cannot be considered as independent data points in statistical analyses (Felsenstein 1985; Harvey et al. 1991b; Martins et al. 1991; Garland et al. 2005). There are several methods to take phylogenetic information into account and investigate statistical relationships among characters (discrete or continuous) or between characters and environmental variables (Felsenstein 1985; Grafen 1989; Maddison 1990; Garland et al. 1992; Pagel 1992; Pagel 1994; Martins et al. 1997). Three most commonly used are: independent contrasts, generalized least-squares models, and Monte Carlo computer simulations, which can be applied to a wide range of analyses, including correlation, regression, analysis of variance and covariance, and principal components analysis (Felsenstein 1985; Harvey et al. 1991a; Martins et al. 1991; Garland et al. 1992; Garland et al. 1993; Garland et al. 2000; Rohlf 2001; Garland et al. 2005). Because it emphasizes correlations between multiple independent evolutionary events, it is possible elaborating and testing generalizations of nearly universal attributes of life, for example, the evolution of brood parasitism, mating systems, sexual dimorphism, velocity and optimal physiological temperature or metabolism and diet (Huey et al. 1987; Garland et al. 1991; Moller et al. 1994; Moller et al. 1995; Owens et al. 1997; Martin et al. 2000; Cruz-Neto et al. 2001; Dunn et al. 2001; Kruger et al. 2002). In summary, the convergence approach hypothesis is that the appearance of a trait among unrelated species in response to similar extrinsic selection regimes in similar,

but spatially distinct habitats (Miles et al. 1993) or the evolution of specific traits combinations across taxa, have major effects on fitness and hence both cases are favored by natural selection.

Trend approach

In spite of this is the most ancient of the phylogenetic approaches to assess evolution by natural selection; analytical methods to evaluate phylogenetic trends have been developed slowly. Traditionally, evolutionists, e.g. Darwin (1859), interpreted morphologic trends as evidence of pervasive natural selection sorting individuals within species (Wagner 1996). This could be the most illustrative way in which Darwin's image of the constant, directional effect of natural selection could generate cumulative changes that produce forms difficulty explainable by chance. Chance does not would privilege particular directions over long-term process and by so long times. One of the most scrutinized trends is that noted by E. D. Cope (1871), regarding the size increase in mammalian fauna during the Cenozoic (Carroll 2001). Cope attributed the pattern to an active trend towards increasing size, which has been attributed to natural selection favoring larger sizes, for example, for evasion of predators, increased brain size or increased longevity (see Stanley 1973), but rigorous evidence for such a trend was lacking (Carroll 2001). Stanley (1973) proposed that Cope's rule could be due to the tendency for new groups to evolve at small size, that is, an initial minimum value, such that there is a passive drift towards larger mean body size in descendants through an increase in variance. Thus, the first question for the study of trends by paleobiologists is that the existence of passive trends, that is, the increase in the total

variation exhibited within a clade, with some constraint or lower bound on potential morphologies making the increase unidirectional, must be ruled out to accept the existence of an active trend (Maynard-Smith 1970, Fisher 1986, Gould 1988, 1990, 1996, McKinney 1990, McShea 1993, 1994, 2000, Wagner 1996, Jablonski 2007). For example, Gould (1996) argues that any recognizable trend can be explained by the "drunkard's walk" model, where the trend is due simply to a fixed boundary condition. Likewise, large-scale trends could be passive with constraint as underlying cause. For example, the number of cell types in a eukaryote cannot be less than one, and this mathematical (or logical) constraint imposes a lower bound on the number of cell types in metazoans (McShea 2005). Valentine et al. (1994) suggested that the increase in number of cell types over the history of animals may have been passive in this way. The simplest rule to recognize an active trend is the biased *replacement* of pleiomorphic morphologies by derived ones (Wagner 1996), but more strict statistical methods have been proposed to distinguishing between active and passive trends (Wang 2001, 2005). Active trends corroborating the Cope's rule has been founded by MacFadden (1986) in the evolution of body size of horses within this era and Alroy (1998) in an analysis of body mass estimates for fossil mammal species, however, it has not found neither Cretaceous mollusks (Jablonski 1997) nor planktonic foraminifera (Gould 1988). Active trends for other characters have been found too, indicating that active trends do operate within overall passive trends (see references in Carroll 2001).

A second and more difficult question is the mechanism behind an active trend. In addition to the organismic natural selection, it has been proposed that active trends could be driven by the differential speciation and survivorship of entire species, or both (Eldredge &

Gould 1972, Stanley 1975). If this is the case, a single prediction, called the "Wright's rule" (Wright 1967) should be satisfied: ancestor-descendant transitions occur in either direction along a morphologic gradient with equal probability (Gould 1977, Gould & Eldredge 1977, Wagner 1996). Thus, if morphologic changes from ancestor to descendant are indistinguishable from 50:50 with respect to the overall trend, differential speciation/extinction cannot be ruled out. The mentioned study of Alroy (1998) found a consistent increase in body size between matched pairs of younger and older species. Nevertheless, other possible causes for active trends can underlie patterns of active trends. For example, restriction on morphologic evolution permitting the evolution of particular morphologies but discouraging the loss of those morphologies (Gilinsky 1981, Shcindel 1990), or differential diversification within a clade (whether by chance or selective species sorting) based in other character, can result in trends for characters that are associated either biologically or phylogenetically (Wagner 1996). The number of alternatives mechanisms explaining active trends (Wagner 1996, Jablonski 2007) makes difficult to prove that natural selection (at the individual level) is the sole cause of the trend. For example, Wagner (1996) proposed tests to distinguish these possible mechanisms from the natural selection at the individual level, i.e. the Darwinian explanation, but the results in morphology of gastropods showed that several of these mechanisms seem underlie some active trends (Wagner 1996). The increase in septal-suture complexity in Paleozoic ammonoids may have been a trend driven with selection (McShea 2005). Saunders et al. (1999) documented that suture complexity increased significantly more often than it decreased among lineages, and they speculated that selection may have been the cause of this upward bias. Nevertheless, more generally, any large-scale driven trend in complexity

could be interpreted as a spontaneous non-selectively mediated process of increasing of complexity, with or without reinforcement by selection (McShea 2005).

These methods are not directed to rule out the possibility of that the morphologies attained in the active trend are not attainable by chance. In fact the range of morphologies and complexity attainable by a passive trend or spontaneous no-selectively active trends are, in principle, on the range of the selection-driven active trend (Gould 1996, 2002, McShea 2005). Nevertheless, trend approaches could be used to test patterns usually adjudicated to chance by phylogenetic character mapping. An example is a recent study to test in nematode the phenomenon known as "developmental system drift", that is, the surprising amount of developmental variation observed for otherwise highly conserved features (Kiontke et al. 2007). Developmental system drift has been commonly explained by stochastic processes (e.g., drift and absence of selection-dependent constraints), but Kiontke et al. (2007), based on the number of convergences and reversals in the phylogenetic mapping, concluded that selection and not stochastic process, such as random drift, explain the results. The parsimony methods used by Kiontke et al. (2007) to assess the reversions has been criticized (Collin & Miglietta 2008), but recent advances have improved the possibility to test statistically this type of hypotheses of character evolution (reviewed in Collin & Miglietta 2008). It is important also to consider that some trends could be due to that the loss of complex features in evolution tend to be irreversible (the Simpson's (1953) version of the "Dollo's law"); thus loss characters cannot re-evolve because the genetic and/or developmental features underlying that unexpressed character accumulate mutations that are extraordinarily unlikely to be reversed (Strathmann 1978, Gould 2002, p. 729, McShea 2005, Collin & Miglietta 2008). The type of trend interesting

to the natural selection creativity depends on the gaining of traits and complexity unlikely to gain by chance, thus trends that are instances of the Dollo's law (e.g., Igic et al. 2006) must be ruled out, because the loss of complex characters is probably due the neutral accumulation of deleterious mutations and not due natural selection (but see Collin & Miglietta 2008).

DISCUSSION

What genetic tests of adaptation do and do not prove?

The absence of evidence for adaptation at the genotypic level was apparent around the 1980: "It has been proved remarkably difficult to get compelling evidence for changes in enzymes brought about by selection, not to speak of adaptive changes" (Lewontin 1979). Paradoxically, the current support for increasing cases of selection in molecular evolution (e.g., Fay et al. 2002, Bierne et al. 2004, Eyre-Walker 2006, Tang et al. 2007, Bachtrog 2008), come from tests that utilize the neutral model as the basis to make the null hypothesis, a model originally proposed to negate that selection is dominating, even relatively important, to explain molecular evolution (Kimura 1983).

Several genetic tests of natural adaptations search single molecular targets of positive selection (e.g., the locus of a single selective sweep). In this sense, they look for historical molecular adaptations in the sense of the "frequency hypothesis". Others genetic tests seek the pervasive presence of natural selection fixing several mutations in specific loci or genomic zones (e.g., the tests based on differences between classes of mutations). In some sense, these studies indirectly bring near to the "origin hypothesis", because the

fixation of mutations by natural selection actually can be understood as "cumulative". Nevertheless, it is not known if there is some functional or general phenotypic relationship between the mutations fixed by natural selection. This problem is inflated because, in most of the tests, the effects of specific sites of mutations fixed by natural selection are not known. That is, it is not known if this "cumulative selection" has some direction related to a specific trait of functional phenotype (see below for exceptions). Probably this fact is in part explainable because the origin of the use of the tests was in the neutral-selection controversy, which refers to proportion of evolutionary molecular changes explainable by drift or selection (Nielsen 2005). Besides, the neutral theory made emphasize in the distinction between molecular and phenotypic evolution (King & Jukes 1969, Kimura 1983, Beatty 1992). Accordingly, none of those tests focuses detecting specific historical cumulative adaptations, but search the relative proportion of positive selection and random drift as evolutionary forces or specific sites of single positive selection processes.

On the other hand, if the pervasiveness of advantageous mutations fixed by natural selection were proved, this could be understood as a strong presumption for the origin hypothesis, even whether it is not known the specific phenotypic effects of single substitutions. Nevertheless, it has been argued that such positive selection might not signal of increased adaptation, but signal of natural selection maintaining functions, that is, simply upholding the *status quo* (Hartl & Taubes 1996, Kondrashov et al. 2002, DePristo et al. 2006, Pal et al. 2006, Camps et al. 2007). For example, mutations fixed by NS can be mostly compensatory mutations that suppress deleterious mutations fixed by random drift (Hartl & Taubes 1996, Kondrashov et al. 2002, Pal et al. 2006), or suppress pleiotropic effects of other favorable mutations (De Pristo et al. 2005, Camps et al. 2007). Recently,

two studies undermine the assumed connection between fast evolution and pervasive positive selection, showing that apparent hotspots for positive selection have probably accelerated evolution by means of a biased DNA repair process, that is, not positive but probably detrimental changes (Berglund et al. 2009, Galtier et al. 2009). Thus, speedy evolutionary change measured either by d_N/d_S and MK criteria is giving a false signal of positive selection (Hurst 2009), where part of the high rate of evolution could be because of the subsequent spread of compensatory mutations (Galtier et al. 2009). Another serious problem of these tests is that speedy molecular change might be plausibly interpreted as evidence of the relaxation of purifying selection (Hughes 2007). For example, rapid evolution of primate microcephalina is interpreted as statistical evidence that it has played a role in adaptive evolution of brain size in hominids (Wang & Su 2004), but might just as plausibly be interpreted as evidence of a reduction in the importance of microcephalin in the higher primates, that is, the extremely opposed interpretation (Hughes 2007). Thus, an essential step in the studies of adaptive evolution is correlating patterns of nucleotide substitutions with questions of protein function and phenotypic effects of mutations (Hoekstra & Coyne 2007, Hughes 2007, 2008). Therefore, genotypic tests of natural adaptations could be necessary but not sufficient to address the origin hypothesis by themselves.

What phenotypic tests of adaptation do and do not prove?

Phenotypic tests of natural adaptations work in the key level of analysis for the origin hypothesis, which ask for the origin of phenotypic observable traits. Nevertheless, textbook

traditional examples of the natural selection action at the phenotypic level, e.g. the contemporary microevolutionary studies of the melanic moth *Biston betularia* (Kettlewell 1973) or the Darwin's finches (Grant 1999), show reversible changes in the frequency of already observable phenotypic traits. Moreover, the genotypic changes underlying trait frequency changes are about the standing genetic variation, although the genetic of the traits is not well known yet (e.g., see Abzhanov et al. 2008). It has been recognized that the evolutionary change on morphological traits is very difficult to connect with gene frequency change except in relatively few circumstances (Ohta 1992). Despite a long quantitative genetic tradition that emphasizes the significance of standing variation, we do not know if most long term evolution (yielding fixed species differences) has much to do with such variation (Orr 2001, 2005a, 2005b). For example, a substantial portion of standing phenotypic variation for *Drosophila* bristles, reflects transposable element insertion polymorphisms, which do not appear to often contribute to species evolution (Long et al. 2000, see also Khaitovich et al. 2006, Hoekstra & Coyne 2007). This could be in coherence of the King's and Wilson's (1975) hypothesis that major morphologic changes in evolution are due to regulatory elements, a central statement of the evolutionary developmental biology (evo-devo) research (Gilbert et al. 1996, Carroll 2000a, 2000b, 2005a, 2005b). However, while King and Watson (1975) emphasize the small number of regulatory changes producing major developmental effects (see also Ronshaugen et al. 2002), Carroll (2000b) argues that regulatory elements does not need dramatic macromutations to play significant role in morphological evolution, based in the apparently abundant variation in regulatory regions in natural populations. Unfortunately, studies supporting the adaptive significance of regulatory regions do not known if one or several mutations were required (Wray 2007). Several studies that mapped the genes contributing

to quantitative variation founded that individual loci account for a surprisingly large amount of the total variation (de Belle et al. 1989, Mackay and Langley 1990, Doebley & Stec 1991, 1993, Bradshaw et al. 1995, 1998, Liu et al. 1996, True et al. 1997), and the large effect was attributed to a single mutation (Stern 2000). Nevertheless, these studies do not resolve the detailed of mutations implied and remains possible that the observed effect are the product of multiple small-effect mutations (Stern 2000). Although loci of large effect continue being commonly founded in several quantitative studies (Tanksley 1993, Falconer & Mackay 1996, Kearsey & Farquhar 1998, Orr 2001, 2005, Mackay 2001), no general results about the number of mutations responsible for these large effects loci exist.

New traits (single or compound, *sensu* Budd 2006) can arise during the anagenetic change of species, during a cladogenetic event of speciation or throughout longer lineages involving many speciation events (Gould 2002). All these processes involve long-term evolutionary processes and it is entirely possible that a good deal of long term evolution involves the fixation of new mutations (Orr 2005a). Thus, it is necessary to put in relationship the contemporary phenotypic studies of natural selection with information at the genetic level scoping long-term processes. Regrettably, the correlational approach cannot distinguish if the continue response to selection is due to standing genetic variation or due to new mutations, a problem difficult to resolve even for artificial selection processes (Barton & Keightley 2002).

One of the longest running debates in evolutionary biology concerns the sufficiency of processes observed within population and species for explaining long-term evolution (Pigliucci & Schlichting 1997, Carroll 2000b). Besides, studies of phenotypic studies of quantitative genetics are difficult to extrapolate to long-term dynamics (Barton & Turelli

1989, Pigliucci & Schlichting 1997, Carroll 2000a). For example, the strength of selection in natural populations decreases when is measured over longer intervals of times (months and years compared with days) (Hoekstra et al. 2001), then probably the strength of selection could differ even strongly for longer evolutionary scales of time. Furthermore, it is well known that trait heritabilities can vary with environmental conditions and with changes in gene frequency (Hoffmann & Merilä 1999, Wilson et al. 2006) and the same occurs for the genetic correlations (Sgrò & Hoffmann 2004). Although rapid rates of morphological change has been observed from generation to generation as a result of rapidly changing selection coefficients (Grant 1999), unidirectional selection is rarely maintained for a sufficient time to result in continuing morphological or physiological modification (Carroll 2000a). Over the duration of most species, the intensity and direction of selection change repeatedly, either in an oscillating manner or in what appears to be a random walk (Carroll 2000a, see also Wilson et al. 2006).

Most of adaptation tests prove that deterministic processes are the more important cause of population dynamics of trait's frequency (with the undeterministic genetic drift null hypothesis). That is, most of the tests are directed to test if natural selection explains the frequency change of traits in nature (why trait frequencies are that they be), but other theme is that natural selection explains the forming of traits in nature (why individual organisms have the traits they do). Accordingly, it has increased the recognition that the origin of traits cannot be assessed only by the traditional population genetics studies centered in quantitative variation of contemporary species, and that developmental and phylogenetic criteria must to be incorporated (Arthur 2000, Newman & Muller 2000, Newman 2005, Muller & Newman 2005, Budd 2006). Also it has been recognized that

long-term dynamics cannot be understood from phenotypic analyses of quantitative genetics, and requires more understanding about the genetic of adaptation (Barton & Turelli 1989), an issue particularly poorly understood (Orr 2005b).

What phylogenetic tests of adaptation do and do not prove?

The scale of work of phylogenetic tests has the advantage to cover long-term processes, and both the homology and convergent approaches have good evidence of the NS frequency hypothesis for evolutionary trait transitions. Nevertheless, phylogenetic information cannot resolve the detail about the steps making raise the trait in the phylogenetic transitions. For example, it has been proposed that speciation is the basis of all phenotypic discontinuities (the "punctuated evolution" of Eldredge & Gould 1972), implying that discontinuities do not originate within species. According to this theory the major evolutionary changes occurs by abrupt acceleration in the rate of evolution as a result of speciation (West-Eberhard 2003, p. 11). S. J. Gould was tempted by the saltational (i.e., single mutation, even non-adaptive) explanation of punctuated phylogenetic transitions (Gould 1980a, p.188, 1980b, 1982a, 1982b, see also West-Eberhard 2003, pp.11ff. and ch. 30), but later he recognized that this is not an issue determinable by the phylogenetic information (Gould 1992, see also Dennett 1995, ch. 10). However, the saltatory issue re-emerged because large-step (mainly regulatory) changes are potentially more rapid that small-step gradual change mediated by selection on mutations of small effect (West-Eberhard 2003, p. 12). Thus, neither tests about transitions that coincide with a given selective pressure and consistently show signals of fitness improvement (the homology approach) nor tests about

traits that show convergent transitions in similar environments (the convergent approach), distinguish between traits continuously formed by natural selection than traits originated by single mutations and spread in the species by natural selection. An intermediate position is that the trait structure at initial suddenly emergence would be rough, and was "fine-tuned" by later (Gould 1980a, p. 383, 1982), in such a case NS does not play a creative role in building the key feature (Gould 1982a, p. 383) and the phylogenetic tests would be proving something almost equivalent to the frequency hypothesis.

Trend approaches, however, enables to prove the existence of cumulative processes of transitions in directions driven by natural selection. Thus, if transitions observed were the gradual steps in the formation of a trait improbably attainable by chance, these tests could prove the creative role of natural selection by themselves. Unfortunately, observed trends are about the simple variation of the magnitude of quantitative traits (or body size) or about the number of meristic, serial traits of general measures of complexity (Carroll 2001), and the range of observed morphologies and complexity attainable by a active trends is, in principle, on the range of the passive or no-selective trends (Gould 1996, 2002, McShea 2005). Furthermore, there are no tests developed to discard this possibility. Thus, trends forming traits or body plans by the trans-generational accumulation of changes remains illustrated theoretically (e.g., Nilsson & Pelger 1994, Lenski et al. 2003) but it is still an unsolved issue (Budd 2006).

Probably the major difficulty to prove the creative role of NS by phylogenetic trends is due the challenge of scientists that have negated the creative role of natural selection (Apendix B for a review). For example, according to T. H. Morgan (1932) NS merely preserves certain traits but, in the absence of NS, in addition to the known forms of

life, a vast assemblage of other types would exist which have been destroyed by selection (Huxley 1936, 1943). Thus, according to this viewpoint NS only have a negative or "destructive" role (Arthur 1997, 2000, Mahner & Bunge 1997, Muller 2003, Muller & Newman 2003, Ariew 2003, Reid 2007, Badyaev 2008). Contrary to this position, the mainstream viewpoint about the creative role of natural selection claims that NS is creative because the complexity and intricate functional organization of traits formed by NS is not attainable by stochastic processes (Huxley 1942, Simpson 1947, Mayr 1963, Ayala 1970, Dawkins 1986, Maynard-Smith 1989, Gould 2002, Ayala 2007, Avise & Ayala 2007). Observed NS-driven trends about quantitative change in size or number of already existent traits cannot rule out that they could be attainable by stochastic and non-adaptive processes. In fact, for trends on complexity, (negative) selection could be proposed if no trend in the mean occurred, but if mean complexity increases, and if the trend is ultimately found to be largely driven, it would still be impossible to say whether the trend was occurring with the support, neutrality, or partial opposition of selection (McShea 2005).

The integrative approaches

The previous discussion show that neither the genotypic, phenotypic, nor phylogenetic tests of natural adaptations can by themselves to test the origin hypothesis about the NS, and so the creativity of natural selection in the formation of traits. Fortunately, integrative approaches have emerging that can assess the evolutionary trait formation more directly. Two groups of approaches are emerging, one under the terms "paleomolecular biochemistry" (reviewed in Golding & Dean 1998) or "functional synthesis" (reviewed in

Dean & Thornton 2007), the other under the terms "evo-devo synthesis" (Gilbert et al. 1996), "eco-evo-devo" (Jablonski 2007), "new synthesis" (Gilbert et al. 1996, Carroll 2000a), or simply "mixtures of phylogenetic and functional analyses" (Budd 2006). Typically, these approaches involve the use of genotypic, phenotypic and phylogenetic approaches together.

In the first group, the main goal is to reveal how ancient mutations altered biochemical processes and produced novel phenotypes (Dean and Thornton 2007). Genotypic tests are used to localize possible targets of natural selection. Phylogenetic analyses are used to infer the ancestral molecular sequence of the putative adaptive genes. Single amino-acid replacements are reconstructed by manipulative molecular techniques (from here the "paleomolecular biochemistry"). Molecular analyses of gene action and manipulative experiments are used to characterize the maps that relate changes in gene sequence to changes in phenotype and fitness, revealing the mechanisms by which specific mutations produce new phenotypes (Dean and Thornton 2007). Throughout these procedures each mutation is accompanied by a description of its effect on protein function and the evidence supporting the causal link between mutation and phenotype, and in some cases the adaptive nature of the change (see Table 1 of Hoekstra & Coyne 2007). For example, Hoekstra et al. (2006) identified a single amino-acid replacement that confers an adaptive change in the coat color of the beach mouse (*Peromyscus polionotus*), and Yokoyama et al. (2008) identified 12 amino acid sites in the rhodopsin at which changes occurred in the evolution of color vision in vertebrates, favoring shifts toward the wavelength of maximal sensitivity around the light spectrum in the dim-light environments. Generally, substitutions with an effect that tend to the derivate phenotype are considered

single advantageous substitutions. Notably, the number of these advantageous substitutions usually is only one (Hughes 2007) or less than a dozen (Hughes 2007, Dean & Thornton 2007, see Table 3 of Nei 2005 and Table 1 of Hoekstra & Coyne 2007).

This approach seems to be the most ambitious research program to face the empirical evaluation of the mechanisms by which genetic change would produced new phenotypes, and thus seems to be the most suitable approach to assess the origin hypotheses. The majority of genotypic tests of natural adaptations does not relate the phenotypic effect of genes or putative favorable amino acid substitutions with specific phenotypic effect, keeping unclear whether any of the species differences in genes have an adaptive (or even phenotypic) effect (Hoekstra & Coyne 2007). Given that there is an increasing awareness that genotypic tests of natural adaptations are not capable to tackle the problem of the origin of adaptive phenotypes (Hughes 2008), it has been proposed that the functional synthesis approach should will become the minimal standard to propose adaptive evolution for traits (Hughes 2007, 2008). Nevertheless, this approach does not provide properly a test in the statistical sense. In fact, given the low number of substitution found in the functional synthesis studies, the current molecular statistical tests of selection are not applicable for them (Hughes 2007, 2008). This implies that this approach could not necessarily determine the population processes that originally gave rise to those changes (Hughes 2008). For example, even for the exhaustive study of Yokoyama (2008), there was no direct evidence that natural selection was actually involved in fixing adaptive changes, and consequently non-Darwinian processes, as the "Dykhuizen-Hartl effect" (Dykhuizen & Hartl 1980) proposed by Kimura (1983), cannot be ruled out in the explanation of this evidence (Hughes 2008).

In the second group of integrative approaches, evolutionary developmental biology (evo-devo), paleontological and paleoecological information are used together to assess adaptive hypotheses about the evolution of morphological traits. As we discussed previously, an important tenet of evo-devo research program is that adaptive mutations affecting morphology are more likely to occur in the cis-regulatory regions than in the protein-coding regions of genes (King &Wilson 1975, Carroll 2000a, 2000b, 2005a, 2005b, Muller & Newman 2005). Indeed, the emphasis on gene regulation is the evo-devo's most famous and widely accepted contribution to evolutionary theory (Hoekstra & Coyne 2007). The evo-devo methods for determining the role of gene regulation in evolutionary change involve the comparing differences in a phenotype among species with the pattern of expression of a single gene thought to influence that phenotype. As we discussed previously this studies have been criticized because the source of phenotypic differences are not pinpointed (Hoekstra & Coyne 2007). Thus, the common methods of observing spatiotemporal patterns of gene expression and experimentally manipulating protein levels can do no more than show an association of gene expression with phenotype and perhaps implicate the developmental pathway in which the causal mutation lies, but not the number nor locus of phonotypically relevant mutations. That is, this approach does not show if the causal mutation is located in the cis-regulatory region of the protein of interest, or upstream of the gene of interest, and so on (Hoekstra & Coyne 2007). This is because most of the regulatory elements are small, not strictly conserved, and often far removed from the gene, making them difficult to identify and to pinpoint their functionally relevant sites (Wray 2007, Hoekstra & Coyne 2007). Additionally, most comparisons of developmental gene expression and regulation have focused on large differences between groups at higher taxonomic levels (Carroll 2000b), and in this comparison the structural genes usually show

some nucleotide differences, making it difficult to pinpoint the causal substitution(s) are in the regulatory or in the structural gene regions (Hoekstra & Coyne 2007). The 30 years old question of whether regulatory protein changes are more important than structural ones for the phenotype evolution, for example in the human evolution, has been considered to be a fruitless one (Khaitovich et al. 2006). When there is positive selection for novel functions in an organ system, both structural changes and expression changes could occur to bring about a change in phenotype. For example, increased enzymatic activity in a tissue can occur through increased expression of the relevant enzyme or by structural changes that increase enzyme activity (Khaitovich et al. 2006).

The method that is the foundation of the evo-devo approach consist on comparing developmental mutations typically of large effect with inferred evolutionary transitions (Bateson 1894, Goldschmidt 1940, Turrill 1940, Stubbe 1959, Lewis 1963, Hillu 1983, Gottlieb 1984, Whiting & Wheeler 1994, Cohn & Tickle 1999). This laboratory-based view of developmental evolution has been strongly criticized (Raff & Kaufman 1983, p. 339, Coyne & Lande 1985, Stern 2000, Budd 2006). The reason is that the effects of these mutations may provide inaccurate indications to the true evolutionary path. According to Raff and Kaufman (1983) and Stern (2000) such mutations are unlikely to represent evolutionary pathways in reverse, mainly because regulatory proteins acting early in organ specification probably regulate many target genes. Thus, "It is easy to remove the regulation of all of these genes in one step by mutation, but essentially impossible to build up all of the required regulation in few steps" (Stern 2000). Coyne and Lande (1985) call this extrapolation the "mutationist fallacy", "such logic would lead one to ascribe differences in body size between populations or species to a single gene because there are

mutations causing dwarfism" (Coyne & Lande 1985). Therefore, it is concluded that is misleading to extrapolate from mutational effects within a single species, particularly those that produce apparently atavistic changes, to likely evolutionary progressions.

The traits of special interest in the evo-devo researches are the "major novelties" characterizing the origin of major taxa (Jablonski 2007), or "key innovations" characterizing the origin of major radiations (Muller & Newmann 2005). In addition to the new synthesis between developmental and paleontological information (Carroll 2000a), it has been recently incorporated ecological information in an "eco-evo-devo" synthesis (Wake 2004, Jablonski 2000, 2005, Jablonski 2007). One of the patterns found is that major novelties characterizing genera appear to originate preferentially in the tropics (Jablonski et al. 2006) which might suggest that this dynamic prevails at all levels, even for higher taxa novelties (Jablonski 2007). It is necessary to incorporate the ecological information in the evo-devo studies to asses selective hypothesis for the origin of traits, but the patterns founded are not simple to interpret. For example, the end-Cretaceous extinction of various archosaur clades opened up a host of ecological opportunities and dramatic diversifications ensued (Jablonski 2007). Nevertheless, this fact has been used to argument that major novelties occurs justly when selective constraints are relaxed, that is when the negative selection force is weakened (Reid 2007, Badyaev 2008). It is to be noted yet that not all evolutionary novelties meet with immediate success or have immediate ecological impact (Jablonski 2007). Macroevolutionary lags, where origination of a major group or acquisition of a major innovation is followed by a long quiet phase before an upturn in diversity or abundance are quite common in the geological record (Jablonski 2007).

Although the eco-evo-devo synthesis studies address important information about the patter and context of the major traits origin, this information is more able to assess macroevolutionary patterns than the origin of specific traits or adaptations (Jablonski 2007). On the other hand, the functional synthesis assess a more detailed account of the genetic steps underlying the evolution of specific traits, but apparently is more suited for the formation of evolutionarily recent traits than for "major novelties" or "innovations". Nevertheless, although the functional synthesis does not have a strict test to the origin hypothesis about the formation of traits by natural selection, is the most interesting demonstration of the beginning of our capacity to assess the particular steps taken in the evolutionary origin of traits. Besides, it refocuses research on a new agenda, using new tools and revised standards of inference, by which it has proposed that this research program provides ample opportunities for the reunification of biology, even subsuming the evo-devo approaches (Dean and Thornton 2007), and incorporating either model as non-model organisms (Vasemagi & Primmer 2005).

Why to test the creative effect of natural selection?

The creative effect of natural selection (the origin hypothesis) seems to be one of the principal theses of Darwin's *Origin of Species*. In fact, the simple maintaining or spread of an individual variant (the frequency hypothesis) seem a minor problem to him (Gould 2002, p. 164). Indeed, using domestication as an analog, Darwin distinguished the effect of natural selection so:

"If selection consisted merely in separating some very distinct variety, and breeding from it, the principle would be so obvious as hardly to be worth notice; but its importance consists in the great effect produced by the accumulation in one direction, during successive generations, of differences absolutely inappreciable by an uneducated eye (differences which I for one have vainly attempted to appreciate)." (Darwin 1859, p. 32)

We cannot suppose that all the breeds were suddenly produced as perfect and as useful as we now see them; indeed, in several cases, we know that this has not been their history. The key is man's power of accumulative selection: nature gives successive variations; man adds them up in certain directions useful to him. In this sense he may be said to make for himself useful breeds." (Darwin 1859, p. 30)

The importance of this point is expressed by the Gould's (2002) emphasis on the origin hypothesis as an essential statement of the Darwinian project (see also West-Eberhard 2003, pp.11ff.):

"If the variations that yielded evolutionary change were large—producing new major features, or even new taxa in a single step—then natural selection would not disappear as an evolutionary force. Selection would still function in an auxiliary and negative role as headsman—to heap up the hecatomb of the unfit, permit the new saltation to spread among organisms in subsequent generations, and eventually to take over the population. But Darwinism, as a theory of evolutionary change, would perish—for selection would become both subsidiary and negative, and variation itself would emerge as the primary, and truly creative, force of evolution, the source of occasionally lucky saltation" (Gould 2002, p.143)

Either a *Drosophila* bristle involving a single population variation and a vertebrate eye involving many inter-generational changes are traits in a genuine sense (Budd 2006), but the explanation of their origins involves different issues. The major interest of this

review is the explanation about the origin of traits in general, not only the origin of ("large" of "small") single variations. A morphological improvement, a variation of a physiological process, a new or modified behavior or extended phenotype, can be formed by a single mutation in one generation or by multiple mutations throughout multiple generations. Although we do not know the precise series of steps that form traits, apparently the majority of interesting traits for Biology are not structures arising from only a single variation. This could lead to us to think erroneously that the creativity of natural selection is already guaranteed by the fact that traits are complex and probably involve the accumulation of many inter-generational changes. Several lines of theoretical work show this mistake.

For example, the "internal-variance principle" of McShea (2005), assert that a pervasive active trend toward the increase of complexity (measured as the number of type of differentiated parts) is expected from any simple study of formal possibility of changes of any phenotypic structure in evolution. Organisms are expected to accumulate variations spontaneously as they evolve, with the result that their internal parts become more differentiated. In fact, the internal-variance principle describes a tendency for the accumulation of variation to produce greater complexity, prior to any consideration of selection (McShea 2005). This non-selectively driven trend toward increasing complexity is also predicted by Gould (2002) and by previous works that treat morphological evolution as a diffusive or Markov process (e.g., Raup 1977). Although McShea (2005) acknowledges that not all aspects of "adaptive complexity" (Ruse 2003) of organisms would be explainable by this principle, he highlights that complexity is not hard to produce, as is common intuition, but in particular to be easy to generate in development and therefore in

evolution. Because all variants must pass through the selective filter, the specific structures that survive should all be adaptive (McShea 2005). Nevertheless, the origin of complex trait could be mainly given by this type of trends toward increased complexity and NS acting as a negative force discarding a subset of alternative, but which if they would not been prevented the complex features would be raised anyway (as pointed early Morgan 1932).

Furthermore, while adaptation within lineages (anagenesis) represents part of the origin hypothesis, speciation and the generation of new lineages (cladogenesis) is the other part, without which morphological diversity would not be preserved (Hoekstra & Coyne 2007). A version of the internal-variance principle of McShea (2005) at the inter-specific level or species diversity is found in the thermodynamic schools of thought in evolutionary studies (e.g., Wicken 1987, Brooks & Wiley 1988) expressed as an "increase of entropy", or other assertions that the degree of morphological differentiation among species in a group, or their "disparity" (Foote 1997), should tend to increase spontaneously (Ciampaglio et al. 2001). The known "speciation genes", i.e., genes whose divergence in DNA sequence causes hybrid sterility or inviability (e.g., OdsH, Hmr, Lhr, Nup96), show evidence of rapid evolution and the signature of positive selection on mutations in coding regions (see Orr et al. 2004; Brideau et al. 2006), although random drift could have an important role too (Mayr 1963, Orr 2005, Uyeda et al. 2009). Thus, the increasing of diversity and complexity of forms could be directly related to NS, but the genetic of speciation and the genetic of phenotypic species differences are two different issues (Orr 2001), where in the later a little is known and the role of few genes of large effects could be important (Orr 2001).

Throughout speciation events, different species could separately fix mutations randomly or by NS for different functions, and thus increase the complexity and diversity

of a higher taxon constituted by a set of species. Likewise, a lineage could fix different mutations by positive selection for different functions or environmental pressures and along this process, as a by-product, increasing the complexity of organisms. Nevertheless, this does not guarantee that these mutations formed an integrated, compound trait or some coordinate or complex organ, subsystem, or developmental program. Furthermore, functions required in some environment or developmental context can change at a later time and thus relax the selective pressure that maintained the previously fixed mutations for traits or trait variants spread by such function. This shows that isolated fixation of mutations by NS could constitute a contingent, transient aggregate of adaptive substitutions do not constituting a consistent whole. Isolated (positive or negative) selection processes could give the adaptive fashion to complex organisms, but the NS could be not driving cumulative changes in an integrated form. For example, a common illustration of the creative role of NS is the antibiotic multi-resistance of bacteria, which is highly improbable without the selection pressure of antibiotic in the environment (e.g., Ayala 1970). Commonly, examples as the previous ones are homologated to the origin of complex traits as the vertebrates eye (e.g., Ayala 1970, Neander 1995a, 1995b, Walsh 1998). Nevertheless, the same example shows the weakness of the homologation. When an antibiotic is extracted from the environment, the respective antibiotic-resistance mutations probably will be removed by neutral mutation or even negative selection due to the adaptive cost of resistance maintenance (Schrag et al. 1997, Bjorkman et al. 1998, Andersson & Levin 1999). Seemingly, the relaxation of selection pressures on phenotypic structures probably involve in the degeneration and loss of the structure due to neutral and deleterious mutations fixed by drift or selection against its maintenance (Collin & Miglietta 2008). Therefore, the functional integration and common selective pressures are requisites,

as well as the fixation of adaptive mutations not attainable by chance, to the NS creative role on the formation of traits (the origin hypothesis). This fact supports the proposal that functional continuity could be considered an epistemic requisite to delimitate the concept of biological "character", "trait" or "homology" (Budd 2006). Nevertheless, the reorientation of the evolution of an integrated trait due to a change of function, producing a "preadaptation", "exaptation" (Gould & Vrba 1982) or "co-opted adaptation" (Buss et al. 1998) obviously could complicate this philosophical problem.

Apart from the mechanism internal-variance principle, there are at least four other possible scientifically valid and in principle non negligible explanations of the complexity of organismal traits. First, Stoltzfus (1999) proposed a model where complex and intricate traits that "appear to be adaptations" arise, not by the classical model of beneficial refinements but, instead, by a repetition of neutral steps. Recently, Lynch (2007a) defended the unavoidable role of non adaptive forces of genetic drift and mutation for the explanation of a large set of evolutionary phenomena as genomic architecture, gene structure, and developmental pathways (Lynch 2007b). Second, it has been proposed that ordered traits with a coordinated stability and order could arise spontaneously in network regulatory systems like genetic or metabolic systems (Kauffman 1991, 1993). Thus the central regulatory and organizational features of cell types and cellular differentiation could be the spontaneous result of systemic relations in networks that meet certain basic features, or the by-product of the increasing of complexity (e.g., the increasing of gene number, Kauffman 1991, 1993), which in turn could be merely due to passive trends (Wagner 1996, Carroll 2001). That is, if the increasing of gene number is a passive non-adaptive trend, and if this is the cause of the major organization of cell types (Kauffman 1991), then NS is not

relevant to the explanation of the major organization of cell types at all. Third, a similar spontaneous tendency to the order is characteristic of thermodynamic systems permanently far from equilibrium (Prigogine & Stengers 1984). Then, because living beings are systems thermodynamically far from equilibrium they have inherently ordered developmental dynamics that cannot be neglected (Brooks & Wiley 1986, p. 23). In fact, the ordered structure and function associated to life are usually considered synonyms of low entropy levels and metabolism is considered addressed to maintain the order by exporting excess entropy (Penzlin 2009). Recently, it has been demonstrated that the compartmentalization associated to the transition from prokaryotic to eukaryotic cells involves a significantly decrease of entropy of living cells (Marín et al. 2009). Coincidently, this and other transitions producing compartimentalization via endosimbiotic processes probably ocurred by a single step in one or a few generations, which can be considered major examples of saltatory evolution (Kutschera & Niklas 2008). Thus, a significantly source of order of eucariotic living beings could be the result of evolutionary processes that not necessarily involve the creative effect of natural selection. Fourth, intracorporeal selection has been historically claimed as an agent of structural and functional phenotypic organization (Gould 2002). Somatic selection (also called "epigenetic selection" or "developmental selection", West-Eberhard 2003) has been proposed as a mechanism that could explain functional patterns without central coordination of elements (West-Eberhard 2003). Emergent functional properties finely tuned by local adjustments could arise by somatic selection, where spontaneous order or self-organization is the consequence of epigenetic selection processes (Kauffman 1993, West-Eberhard 2003, ch. 3). Thus, a good challenge for the origin hypothesis of NS is the suggestion that similar types of traits to the known could

arise according to a neutral model through some of the four previous possible mechanisms for the emergence of organized systems.

The origin of complex traits represent an area of evolution that generate most public skepticism about evolution, and has given most encouragement to creationist objections to evolution as a whole (Gilbert 2003). Virtually all creationist objections to the non-supernatural explanation of complex trait are against the NS, but the difficulty to prove the creative role of natural selection thus is not due to the implausibility that natural selection explains the pattern founded, as creationists claim, but because there several alternative mechanisms difficult to discard (including the commented "Dykhuizen-Hartl effect" (Dykhuizen & Hartl 1980) proposed by Kimura (1983, pp. 270ff.)).

We think that this should not discourage the attempts to making strict tests of the origin hypothesis of NS. As we reviewed, the integrative approaches to the assessment of the evolutionary formation of traits, as never seen before, promise confident information about the sequence of steps carried out in evolution, and this could be the basis to apply future tests to the creative effect of NS. Particularly, current null hypothesis to test adaptations are not directed to demonstrate that the sequence of evolutionary events that originate a complex trait cannot be explained by the random accumulation of neutral changes, one of the most evident alternative hypothesis that must to be discarded. Probably this situation can be explained by historical and conceptual misconception and bias to be mentioned in the following section. The Gould's and Lewontin's (1979) criticism to the "just so" ad hoc adaptive histories of the "panadaptationist" worldview, fixed up to this present moment the statement that the presence of a phenotype does not mean that it was

originated or propagated by selection, and that each hypothesis of adaptation must be tested strictly in terms of specific evolutionary mechanism.

Probably, the creativity of NS in the formation of the pervasive complex traits of organisms is the best available general explanation given the current data and theories. Nevertheless, this is not supported by statistical tests directed to this specific hypothesis and the general superiority of the NS framework could be due in part because the biased amount of studies directed to the adaptionist research program, normally neglecting any other alternative explanation. This is well represented with the lavish adaptationist self-satisfaction like the one of Brandon (1990, p.175): "It is worth noting that presumably no serious biologists think that other evolutionary mechanisms [i.e., other than natural selection], such as drift or pleiotropy, can produce complex and intricate traits that appear to be adaptations."

Why to prove the creative effect of natural selection is so difficult?

In spite of the 1940's neo-Darwinian "modern synthesis" or "synthetic theory" was converted in the mainstream of the evolutionary biology (Huxley 1943, Mayr 1993, Leigh 1999), the development of a mature theory of adaptation has been very slow (Orr 2005b). Even Kauffman and Levin (1987) declared that evolutionary geneticists possess "essentially no theory of adaptation". Orr (2005b) explained the weakness of theoretical development on adaptation by two important historical facts. First, the rising of the neutral theory of molecular evolution. Given that throughout the 1960s and 1970s, evolutionary geneticists increasingly convinced that most molecular evolution reflects the substitution of

neutral or nearly-neutral mutations, not beneficial ones, the study of adaptation itself grew intellectually suspect and the theoretical study of molecular adaptation essentially ceased (Orr 2005b). In fact, the selectionist-neutralist debate was based on a strict differentiation between the phenotypic and the molecular level and directed to the general pervasiveness of selection or drift in molecular patterns but not in specific (molecular or phenotypic) transitions, adaptations, or acquisitions (King & Jukes 1969, Kimura 1983, Beatty 1992). This tendency is strengthen by the fact that the rate of molecular evolution can be decoupled from the rate of phenotypic evolution (e.g., Meyer et al. 1990; Sturmbauer and Meyer 1992), indicating that we cannot extrapolate in a simple way from genotypic to phenotypic variation (Stern 2000).

A second historical cause proposed by Orr (2005b) for the lag in the theory of adaptation was the micromutationist view of quantitative genetics, that is, the assertion that the heritable basis of adaptive evolution was extremely fine-grained (Orr & Coyne 1992, Orr 2005b). The micromutationist assumption enabled to legitimate the study of the process of adaptation without search for concrete genetic changes (Bateson 1902, Orr & Coyne 1992, Orr 2005b). Despite to much confident statements of micromutationists, the theory and the case studies to which these early workers pointed were uniformly and appallingly weak (Orr & Coyne 1992, Orr 2005b). The result is that we have a rich body of mathematical theory on phenotypic evolution, built largely on an infinitesimal foundation, but saying little or nothing about growing body of data on the genetic basis of adaptation, where genes and mutations of different sizes seems to be important (Orr 2005b, see also Stern 2000). Nevertheless, although the mathematical principles underpinning population-level adaptation are being developed (Orr 2005a, 2005b), the origin and evolution of traits

has received far less attention (Budd 2006), and there are many other important questions are not even asked by traditional evolutionary theory (Orr 2005b). Nowadays, rise of molecular methods has led to an increase in the importance of mathematics in population genetics and evolution. According to Crow (2009), the panorama of evolutionary biology has changed drastically from five decades back: "At the time of Mayr's challenge [toward the mathematic methods in 1959], evolution had a beautiful theory but very few opportunities to apply it. Now, the situation is reversed: data appear faster than existing theory can deal with them. That mathematics will play an increasingly important evolutionary role in the near future seems clear." As we have showed, data coming from the functional synthesis could give the empirical basis to go beyond the traditional population genetic models treating the frequency hypothesis, developing models that assess the origin hypothesis. Other historical phenomena that could have been contributed to the deficiency in the assessment of the origin hypothesis, are the twentieth century' divergence in subject matter and scientific culture between evolutionary and molecular biologists (Dean & Thornton 2007), and the conceptual and philosophical limitations of the models of "cause" and "explanation" applicable to the evolutionary biology (see Box 1 and Appendix B).

Beyond the theoretical deficiencies to assess the origin of traits, the complex empirical phenomena involved into the origin of traits reveals the hard nature of the problem. The traditional genetic view of the phenotype invoked a developmental "black box" similar to the one that is still predominant in evolutionary biology (West-Eberhard 2003, Budd 2006). The failure in explain the origin of novel traits made that the structuralist school provided a radical critique of the entire "genocentric" view of complex

character evolution, suggesting that there is a large or even exclusively non-genetic component in how these characters arise (e.g. Seilacher, 1991; Müller & Newman, 1999, 2000, 2003, 2005, Maturana & Mpodozis 2000, Newman 2005). In this view, structures emerge from complex dynamic processes, and then are stabilized by genetic processes (Budd 2006).

A classic alternative explanation of "adaptive complexity" of organisms is the use inheritance Lamarck (1809, Jablonka & Lamb 1995). Contrary to the usual evolutionary textbook assertions, there are several well documented cases of epigenetic inter-generational inheritance (Jablonka & Lamb 1995, 2005). Nevertheless, these instances of Lamarckian inheritance are preserved by only a few number of generations (Jablonka & Lamb 1995), thus probably these inherited changes have poor evolutionary potential (but see Jablonka & Lamb 2005). For "Mendelian" organisms (i.e., those with the inter-generational inheritance systems are linked to the DNA replication) epigenetic or non-genetic structural changes must be genetically "assimilated" to acquire a potential role in the evolutionary process. Thus, strictly non-genocentric changes with evolutionary potential remains mainly as a major possibility in the "pre-Mendelian world" (Newman 2005). The "Baldwin effect" (Baldwin 1896, 1902, Simpson 1953) and the Waddington's (1942, 1953a, 1953b, 1953c, 1961) "genetic assimilation" are possible mechanisms to made potentially inheritable at the long-term those phenotypic changes driven by epigenetic and plastic environmentally induced responses, however they are poorly studied yet (see Crispo 2007 for a review). Overall, both the strictly "non-genocentric" and the "gene-assimilation" approaches have importantly highlighted the relevance of structural characteristics of organisms, both regarding the complex way to incorporate genetic and environmentally

induced phenotypic changes (i.e., "genetic" and "phenotypic accommodation", respectively, *sensu* West-Eberhard 2003, see also Crispo 2007). In fact, there is a variety of types of structural changes that probably organismal systems must to accommodate in the formation of compound traits; all of which must be incorporated in the theoretical and empirical assessment of the "problem of variation" (Stern 2000) and the problem of the origin of complex traits. The structural viewpoint embolden us to recognize that the cumulative evolution is not an additive accumulation of genes, but a sequence of epigenetic changes integrated into previous structures with laws of forms, mechanical and adaptative constraints, developmental and epigenetic programs, in summary, that organisms are complex systems.

The apparently modular nature of living complex systems becomes the assessment of the origin of trait more simplistic and complicated at the same time. Modularity enables to study subsets of systems to understand analytically the whole systems (e.g., Wagner & Altenberg 1996). Nevertheless, the duplication and differentiation of modules or parts (see Gregory 1935; Weiss 1990) could to be important ingredients of the increased complexity of traits (McShea 1991, 2005). Likewise the developmental reorganization via the reshuffling or recombination of discrete phenotypic parts could be an important ingredient of formation of the traits (West-Eberhard 2003). Besides, the loss and recruitment of phenotypic structures could be common in evolution (Collin & Miglietta 2008). New structures and phenotypic traits could recruit not-completely lost modular developmental programs hiddenly maintained by long-term periods by pleiotropy or other causes (Budd 2006, Collin & Miglietta 2008). Moreover, the equivalent process can be pervasive at the genetic level. A commonly cited mechanism for the increasing in genetic complexity is the

Ohno's (1973) theory of gene duplication and random differentiation of duplicated genes that acquire new functions by chance, and it is also proposed that the differentiation or subfunctionalization (Lunch & Force 2000) could be driven by natural selection (Hughes 2002). The importance of this type of genetic evolution becomes strengthened by studies as that one of Demuth et al. (2006) that show that in humans and chimpanzees at least 6% (that is, 1,418 of 22,000 genes) of the genes in one species has no known homologue in the other. This suggests that gene duplication and gene loss occur frequently and contribute to the genetic (and perhaps phenotypic) differences between even closely related species (Hoekstra & Coyne 2007). Therefore, given the potential importance of this mechanisms to the formation of traits, and given the potential importance of non-selective forces as evolutionary forces driving duplicated gene evolution (Ohno 1970, 1973), this provide another reason to not discard the importance of non-selective forces in the formation of traits, and thus in possible tests for the origin hypothesis.

As we has showed, despite the hard complexity and the many difficulties to assess the origin of traits, the functional synthesis begin to provide the specific genetic sequence of changes involved in the evolutionary acquisition of some traits. This type of information gives the exceptional opportunity to prove the creative role of NS in the formation of traits, but theoretical and empirical developments are necessary yet. A pessimistic view about the possibility to test the creative role of natural selection proceed justly from one of the most fervent defenders of natural selection theory: "When one attempts to determine for a given trait whether it is the result of natural selection or of chance (the incidental by-product of stochastic processes), one is faced with an epistemological dilemma. Almost any change in the course of evolution might have resulted by chance. Can one ever prove this? Probably

never." (Mayr 1983). It has to be hoped that this pessimistic viewpoint does not undermine the attempts to resolve one of the most ancient and interesting problems of evolutionary biology.

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Table 1: Classification of tests of natural adaptations according to the level of information used, and the target hypotheses that determine the null model or null hypotheses for each type of test.

Level	Approach	Target of tests	Type of test	References
Genotypic	*Indiscriminate mutation approach*	Differences in allele frequency distribution and/or level of variability	Ewens-Watterson HKA Tajima's D LD-based	Hudson, Kreitman & Aguade (1987), Tajima (1989 – 3?), Fu (1996-5?)
	Type of mutation approach	Differences between classes of mutations within loci	d_N/d_S or K_a/K_s McDonald-Kreitman type PRF (Poisson random field) models	Yang & Bielawski (2000), McDonald & Kreitman (1991), Sawyer & Hartl (1992), Nielsen (2005)
Phenotypic	*Correlational approach*	Presence and response to fitness differences	Trait-fitness population correlation Heritability	Lande & Arnold 1983, Endler 1986, Lynch & Walsh 1998, Kingsolver et al. 2001
	Experimental approach	Ecological explanation of fitness differences	Manipulative experiments Optimization models	Parker & Maynard Smith 1990, Sinervo & Basolo 1996, Dugatkin 1998, Clark & Mangel 2000
Phylogenetic	*Homology approach*	Coincidence between selective regime and trait arising – Exaptation refutation	Coincidence trait-environment Subsequence trait-environment	Coddington 1988; Baum & Larson 1991; Stromberg 2006
	Convergent approach	Trait-environment interspecific correlation	Comparative methods	Huey & Bennett 1987; Blackledge & Gillespie 2004; Martins 2000
	Trend approach	Evolutionary trends - Directionality in evolutionary transitions	Paleobiology Character mapping	Wagner 1996, Kiontke et al. 2007

BOX 1:

Seemingly, Muller (1929) was the first to expose explicitly the creative role of natural selection (NS), stressing the ability of NS to make more probable types of sequences that seems impossible to occur by the random accumulation of changes. This "creative force" of NS has been associated to the NS ability to explain or cause the origin, formation, generation or building of traits (Huxley 1942, Simpson 1947, Mayr 1963, Ayala 1970, Dawkins 1986, Gould 2002, Ayala 2007, Avise & Ayala 2007).

That NS can "explain" or "cause" the origin of traits, makes sense by the *Probabilistic Causation* concept (Reichenbach 1956, sec. 23, Suppes 1970, c. 2), henceforth PC, and the *Statistical Relevance Model of Scientific Explanation* (Salmon 1971, 1984; Jeffrey 1969; Greeno 1970), henceforth SRM. Essentially, according to the SRM an attribute or factor A will be explanatorily relevant to another attribute B iff $P(B \mid A) \neq P(B \mid non\,A)$, that is, iff the probability of B conditional on A is different from the probability of B conditional on the absence of A. On the other hand, the PC in its most sophisticated version, the *Test Situations Theory* (Cartwright 1979, Skyrms 1980, Eells 1991, c. 2, 3 y 4, Hitchcock 1993), asserts that A causes B iff $P(B \mid A\,\&\,T) > P(B \mid non\,A\,\&\,T)$ for every test situations T. A test situation is a conjunction of factors that can be conditioned to be held fixed in order to control its influence on the probabilistic relations between A and B.

Based on these concepts, it is possible to understand the explanatory and causal role of natural selection in probabilistic terms (see Razeto-Barry & Frick 2009). If a trait C is formed from an ancestral structure C_0 by a single mutation m_1, then NS is not explanatory nor causally relevant in the C formation, because:

$$P(C_0 \rightarrow C \mid m_1 \,\&\, NS) = P(C_0 \rightarrow C \mid m_1 \,\&\, non\,NS)$$

That is equivalent to the well-known fact that the probability of mutation is independent of its adaptive effect (Lenski & Mittler 1993). Nevertheless, when the origin of a trait C involves more than one mutation ($m_1, m_2, ..., m_n$), the probability of this type of *sequence*, with and without NS, must be compared. It is possible to demonstrate (Appendix B) that in this case:

$$P(C_0 \rightarrow C \mid m_1 \cdot m_2 \cdots m_n \,\&\, NS) > P(C_0 \rightarrow C \mid m_1 \cdot m_2 \cdots m_n \,\&\, non\,NS)$$

Thus, the causal role of NS into the origin of traits can be understood as the probabilistic causation of the sequence that forms them.

SECCIÓN 2

The creativity of natural selection and the accumulation of advantageous substitutions by chance

ABSTRACT: A long-term argument in evolutionary biology is that natural selection can be creative in the formation of complex traits because the combination of cumulative intergenerational changes required for the formation of a complex trait is not attainable by chance. Generally the possibility of adaptation by chance is discarded and the creative effect is thought guaranteed if trait formation involves two or more advantageous fixations. Thus, only single large mutations forming the trait in one generation guarantees that natural selection is not necessary for trait formation. Here we explore the relationship between the size and number of mutations and the creativity of natural selection. Furthermore, we investigate the possibility that adaptation could occur in a stochastic scenario through the substitution of advantageous mutations by chance, a phenomenon that we called "serendipitous adaptation". Throughout simulations based in Fisher's geometrical model of evolutionary adaptation we found that trait adaptation in a stochastic scenario is not negligible for low number of dimensions, i.e. for traits that involves a few number of independent phenotypic features. This result is inflated when random environmental changes are incorporated into the model. Also it is proposed a heuristic null model to test the creative effect of natural selection trough the proportion of advantageous/(advantageous+deleterious) substitutions. Although this is only a heuristic method, to our knowledge this is the first study attempting to make the creative effect of natural selection assessable. Data coming from the recent "functional synthesis" is currently

available and can be used in order to detect the specific advantageous mutations involved in the evolution of a trait. This information could be the ground to apply this type of tests.

KEY WORDS: Creative role of natural selection, macromutationism, macromutationism, serendipitous adaptations, size mutation effect, number of mutations.

INTRODUCTION

Evolutionary gradualism assumes that evolution occurs by a large number of small genetic variations, whence evolutionary gradualism is also called "micromutationism". Charles Darwin maintained that "natural selection can act only by the preservation and accumulation of infinitesimally small inherited modifications" (Darwin 1959:95) and he was a strong opponent to the idea of confering importance to the large size (phenotypic) changes, as emphasized by the "saltational" or "macromutational" theories (Darwin 1959: 101-102, 132, Orr & Coyne 1992, Gould 2002, Stoltzfus 2006). Although it is not evident why Darwin was so strict about this viewpoint point ("*natura non facit saltus*"= "nature does not make leaps", Linnaeus 1751), it has been proposed that the principal incentive for gradualism is Darwin's conception of natural selection as a creative force (Gould 2002:60-61,140,149-150,343,406). Natural selection can be creative in the origination of a trait when its evolutionary formation requires a combination of new genes or mutations (Mayr 1963, Ayala 2007), that is, when the evolutionary origin of a trait requires a selective factor that make probable combinations of mutation fixations and that otherwise it would effectively impossible to obtain by chance (Simpson 1947, Mayr 1963, Gould 2002, Nanay 2004, Ayala 2007, see Apendix B for a review).

Models under the micromutational framework, as the infinitesimal model of Fisher (1930), assume that evolutionary processes are given by an infinite supply of very small mutations (Coyne & Orr 1992, Orr 1998). It has been claimed that this assumption guarantees that mutations plays little or no creative role in evolution and that natural selection acts as the sole source of creativity in evolution (Turner 1985, Coyne & Orr 1992, Orr 1998). The very contrary position is the "strong" version of macromutationism (*sensu*

Charlesworth 1990), exemplified by the extreme saltationism of Goldschmidt (1940), which maintain that single mutations produce large size, complex adaptations in an essentially perfect form (Orr & Coyne 1992). If this is the case, the origin of traits is not related to natural selection at all given the well known fact that mutations are random, i.e., they do not depend on their adaptive or selective effects (Lenski & Mittler 1993). Therefore, in this case the only creative cause for the formation of the trait is the (macro)mutation itself (Gould 2002:143).

All of these claims lack a formal support and there is not a criterion used to quantify the natural selection creative effect and/or to indicate the relationship between number and size of mutation effects and the creativity of natural selection. It has been proposed that the creative role of natural selection in the formation of a trait is certain when two advantageous mutations are involved (Huxley 1942, Ch. VII(7), see also Neander 1995a, 1995b, Brandon 1992:180). Thus, according to this "two-mutation threshold", the clear cut point that guarantees the creative role of natural selection in the origin of a trait is given when the evolutionary formation of a trait depends on two advantageous mutations. The assumption here is that any trait that involves two or more advantageous mutations cannot be formed by chance (i.e. by random mutation and drift).

An evident flaw of this argument is that it does not contemplate the possibility that two or more advantageous mutations could be fixed by simple chance (random drift). Given that the substitution process is partially determined by stochastic processes in finite populations (Ewens 2004), the probability of mutant fixation of one or more beneficial changes by drift is not zero in finite populations. This fact becomes more important from recent evidence showing a high proportion of advantageous substitutions fixed in evolution -around a 50%

and more (Fay et al. 2002, Bierne et al. 2004, Eyre-Walker 2006, Bachtrog 2008). On the other hand, in finite populations under selection the probability of fixation of deleterious mutations is not zero and increases exponentially with the decrease of population size (Crow & Kimura 1970), and after deleterious substitutions, further advantageous compensatory mutations will probably be fixed with high frequency (Poon & Otto 2000). Thus, both the fixation of advantageous and deleterious mutations by drift is probable in small size populations where drift is the dominant evolutionary force.

In this study we modeled the evolutionary process in the Fisher's (1930) geometrical model (hereafter FGM) in order to assess the creative effect of natural selection with different sizes of mutational changes. We follow Fisher's original proposal of the model (Fisher 1930, Orr 1999) where, a Cartesian multidimensional space can be interpreted as a number of phenotypic changes that may modify a single trait or organismal phenotype (i.e., a trait influenced by a relatively close interacting number of universally pleiotropic phenotypic changes). Thus, in the FGM we define a complex trait as a point with n coordinates into a multidimensional space as the evolution of a single complex trait could involve many phenotypic dimensions: "The representation in three dimensions is evidently inadequate; for even a single organ, in cases in which we know enough to appreciate the relation between structure and function, as is, broadly speaking, the case with the eye in vertebrates, often shows this conformity in many more than three respects" (Fisher 1930:39). Hence, the probability of a population acquiring a trait with high fitness by chance can be faced in the FGM as the problem to reach a point with a high fitness value - like in a selective regime- but only by random mutation and drift. The reader should note that there are several interpretations of the number of dimensions in the FGM, for example,

Gu (2007a, 2007b) represents dimensions as the number of phenotypes affected by the pleiotropic effects of a protein. Thus, our results also could be valid if a protein is considered as a trait by itself.

Furthermore, we modify the usual assumption in the FGM of an absolutely stable environment with a fixed optimum, which is a very unrealistic scenario (Orr 1998, 2005), by incorporating environmental variability. Recently environmental variability has been incorporated into the FGM to model protein sequence evolution (Gu 2007). It is expectable that fitness distributions are modified due to the environmental variability, thus, we also study the effect of environmental variability over the results varying the amplitude and timing of random optimum shifts.

THE MODEL

Simulations

Simulations were developed under the FGM framework (Orr 1998) with the incorporation of deleterious and compensatory substitutions. A trait or trait state is represented as a point into the multidimensional space of phase. Each axis represents an independent phenotypic property of the trait. Simulations were made by Monte Carlo methods where random vectors are generated into a n-dimensional space of phase, with a uniform distribution of vector magnitudes (from 0 to r) (following Kimura 1983, Orr 1998). These vectors represent mutations that change the trait and that can be fixed into an asexual population according the probability function (Crow and Kimura 1970, see Fig. 1):

$$p(N,s)=\frac{1-e^{-2s}}{1-e^{-2Ns}}$$

, where N is the effective population size and s the selection coefficient (Fig. 1a). A low rate mutation is assumed in the model such that at any one point in time, each population is genetically homogeneous. Thus, the number of mutations that occurs per generation is $N\nu$, where ν is the rate of mutation per individual summed over the loci controlling the trait.

Fitness values were determined by the Gaussian function $w(z) = e^{-\frac{z^2}{2}}$, where z is the distance to the optimum point. Selection coefficients are defined as $s = \frac{w_{mut} - w^+}{w^+}$, where w_{mut} is the fitness of the mutant and w^+ is the fitness of the wild-type. We obtained the probability distribution of possible values of fitness of the traits under different evolutionary regimes and different sizes of mutational effects (r). Following the Tachida's (1991) criterion (Ohta 2007), a drift-dominated regime could be represented by a population with small population size (N =10 in our simulations) that satisfies the condition $2N\sigma_s < 0.2$ and a selection-dominated regime could be represented by a population with large population size (N =1000 in our simulations) that satisfies a condition of a selective regime $2N\sigma_s > 3$, where σ_s is the standard deviation of the selection coefficients.

The first set of simulations assume that a recent environmental change lead a trait toward a sub-optimum fitness value given by a unitary diameter with respect to the optimum ($z^+_0 = 0.5$), and the optimum remain fixed the rest of the time. The second set of simulations incorporates permanent environmental changes determined by two parameters that specify its temporality and amplitude, respectively. Theoretical values for equilibrium fitness were obtained from Tenaillon et al. (2007):

$$w_{eq} = (1-(2N-1)^{-1})^{n/2}$$

Optimum can be shifted either by environmental changes or by internal physiological perturbations (Barton 2001, Gu 2007). Temporal variability was simulated as a Poisson process such that $\nu \sim f(\nu,\lambda) = \frac{e^{-\lambda}\lambda^{\nu}}{\nu!}$, where ν and λ are the number and the expected number of changes in a time interval, respectively. Time intervals between consecutive changes (t) follow an exponential distribution $t \sim f(t,\tau) = e^{-\frac{1}{\tau}t}$, where $\tau = 1/\lambda$ is the expected time between environmental changes (pseudo-random numbers were obtained using *expnoise* function available in IgorPro, WaveMetrics, Lake Oswego, OR). The amplitudes of environmental changes were calculated as $a(z_1,...,z_n) = \frac{\sqrt{z_1^2+...+z_n^2}}{\sqrt{n}}$ where z_i are the coordinates of the new optimum which were chosen from a Gaussian distribution centered in the zero point of the Cartesian n-dimensional space, $z_i \sim f(\sigma_a) = e^{-\frac{z_i^2}{2\sigma_a^2}}$, where σ_a represents the standard deviation of amplitudes of environmental changes (we used *gnoise* function available in IgorPro, WaveMetrics, Lake Oswego, OR).

RESULTS

We found very different fitness distributions for drift-dominated and selection-dominated regimes both for stable environments and variable environments (Fig. 2). Nevertheless, distributions with drift and selection-dominated regimes present a significant superposition in range of high fitness values, higher for variable than stable environments (Fig. 2 c, f).

Because the creative effect of natural selection is understood as the capacity of natural selection to carry a trait to a state that is not reachable by chance (see Introduction), superposition areas could be understood as the opposite (or probabilistic complement) to the creative effect of natural selection. Superposition areas could be interpreted as the probability that a drift-dominated process reaches to a fitness value into the range of a selection-dominated process. Thus, the creative effect of natural selection is lower in variable than stable environments (Fig. 2 c, f).

The distribution of fitness values for diverse sizes of mutation effects was plotted for different number of dimensions in Figure 3. We found that with mutations of small size, distribution tends to stay in a neutrally stable equilibrium around the initial point ($w = 0.883$ for $z^{+}_{0} = 0.5$ in the simulations). With a higher size of mutation effects, distributions tend towards a distribution centered on a stable equilibrium state with lower mean for higher dimensions, which is in accordance with theoretical predictions of mutation-selection-drift equilibrium (Fig. 3). In turn, dispersion of distributions is higher for increasing size of mutation effect, thus distributions for higher size of mutations have a higher contribution of fitness values close to the optimum (Fig. 3).

Simulations incorporating environmental variations as random optimum shifts in a selection-dominated regime present a clear pattern where higher temporal variability and amplitude of environmental changes show distributions with a higher proportion of lower fitness values (Fig. 4).

The proportion of advantageous/(deleterious+advantageous) substitutions in a stable environment drift-dominated regime showed a narrower distribution for higher number of substitutions (Fig. 5 a, b). We calculated the critical values for the proportion of

advantageous/(deleterious+advantageous) substitutions to determine a null hypothesis reject zone for a level of significance of $\alpha = 0.05$ and $\alpha = 0.01$ (Fig. 5 e and Table 1). The null model is generated as a drift-dominated model of fixed effective population size. We found that the probability to reject the null hypothesis decreases when the number of substitutions decreases.

DISCUSSION

The substitution of advantageous mutations by chance had not been contemplated until Ohta (and Tachida 1990, 1991) incorporated slightly advantageous mutations to previous models (Ohta 1972, 1973) of nearly-neutral evolution. Nevertheless the nearly-neutral theory continued making strong emphasis on the fixation of slightly deleterious mutations (Ohta 1992, Ohta 1996, Hartl & Taubes 1996; Gillespie 2004:38, Gu 2007a, 2007b). This could be in part explained because these models were strongly criticized (e.g., Gillespie 1994, 1995), and because models usually do not incorporate compensatory mutations. For example, population genetic models that include changes in population size due to mutation fixations predict that many asexual and some sexual populations with low effective population size will inevitable go extinct through mutation accumulation (e.g., Lynch and Gabriel 1990; Gabriel et al. 1993; Lynch et al. 1993, 1995a,b; Lande 1994, 1998; Butcher 1995; Schultz and Lynch 1997). Only recently models have incorporated the possibility that the loss of fitness from the fixation of deleterious alleles could be counterbalanced by a gain in fitness due to compensatory beneficial alleles, something natural into the FGM when deleterious substitutions are permitted (Poon & Otto 2000, Whitlock 2000, Whitlock et al. 2003). Traditionally it has been recognized that the effect of a mutation at

biochemical, physiological, morphological, and behavioral levels may depend on its genetic context (Wright 1968). Nevertheless, the fact that compensatory mutations could be more beneficial in the context of deleterious mutations than when combined with a more fit genetic background has been incorporated only in few models (e.g., Ohta & Tachida 1990, Tachida 1991). This assumption increases considerably the pressure of advantageous mutations in some conditions, amplifying the rate of advantageous substitutions. The experimental work of Lenski and Travisano (1994) and of Burch and Chao (1999) has demonstrated that maladapted populations have higher beneficial mutation rates or higher mean effects of beneficial mutations than well-adapted populations. Thus, advantageous mutations can arise at sufficient rates to allow the recovery of mean fitness even in small populations (Whitlock & Otto 1999, Whitlock 2000). In addition, for lower population sizes not only the mutation rate and mean but also the probability of fixation of advantageous substitutions increases significantly (Fig. 1b). These facts are included as natural assumptions under the FGM, which explains why the substitution of advantageous mutations in a stochastic (drift-dominated) regime is substantial in our simulations. Given the importance of this phenomenon we propose to call it "serendipitous adaptations" (Table 2). However, it is necessary to be careful with such definition, because the role of drift and selection for advantageous mutations is difficult to separate. Mutations are effectively neutral when $|Ns|<1$, i.e. when their fate is mostly governed by genetic drift and not by selection (Kimura 1983). Nevertheless, although this is strictly true for mutations with $s<0$, effectively neutral mutations with $s>0$ are fixed both for drift and selection, being drift much more important than selection until $s \approx 1/N$ (Figure 1b).

It is possible that the creative effect of natural selection had been thought as guaranteed for traits or genes that involve more than one advantageous mutation given the absence of awareness about the probability of substitution of advantageous mutations by chance. On the other hand, the detection of specific mutations responsible for differences between ancestral and derivate phenotypes has been only recently available in a functional synthesis framework incorporating sophisticated integration of genetic, phenotypic and phylogenetic techniques (Dean & Thornton 2007). According to our results the substitution of advantageous mutations in a drift-dominated regime can reach to fitness values that agree with the range of fitness attainable in a selection-dominated regime (Fig. 2). Nevertheless this possibility decreases with the number of dimensions (the degree of complexity) of traits since distributions move away from the optimum (Fig. 3). This is completely coherent with the fact that the expected difference between the equilibrium fitness values of a selection and drift-dominated regime increases for higher dimensions (Fig. 6). Thus, for low dimensionality, it is possible the partial adaptation of traits in a drift-dominated process.

The relation between the size of mutation effects and the role of natural selection in the trait formation remains treated only in a verbal form and it has not been studied systematically. The mainstream Darwinian tradition coming from the modern synthesis (Huxley 1942) makes emphasis on the micromutational position (Orr & Coyne 1996 for a review). On the other hand, recognized critics of the creative role of natural selection have emphasized the importance of mutation of large effects (Gould 2002:143). The structuralist or developmental tradition, ussually in a position opposed to the adaptationist tradition (Gould 1998, Amundson 2001), and the current evolutionary developmental biology (evo-

devo) research program emphasize the role of large effect mutations (King & Wilson 1975, Whiting & Wheeler 1994, Arthur 1997, 2000, Gellon & McGinnis 1998, Cohn & Tickle 1999, Newman & Muller 2000, Ronshaugen 2002, West-Eberhard 2003, pp.11ff., Newman 2005, Muller & Newman 2005, Stoltzfus 2006, see also Hughes 2007). In fact, it has been considered an issue in the evo-devo agenda, principally in the study of regulatory regions, serial elements and changes that do not belong to the constitutive range of variation of a phenotypic precursor (Muller & Newman 2005). Our results indicate that the effect size of mutations is important to determine the distribution of available fitness values (Fig. 3). This result could seem to contradict previous theoretical results predicting that the equilibrium fitness reached by the fixed drift load only depends on the effective population size and the number of dimensions (Hartl & Taubes 1996, Poon & Otto 2000, Sella & Hirsh 2005, Sella 2009). Nevertheless, those studies only contemplate the equilibrium state conditions but not the temporality of the transient necessary to reach to the equilibrium. Although the mutation-selection-drift equilibrium only depends on the population size and number of dimensions, the rate of approach to the equilibrium does depends on selection coefficients (Hartl & Taubes 1996) and therefore on the size of mutation effects. As other similar models with small mutation effects (Ohta & Tachida 1990, Tachida 1991) in our model the rate to equilibrium is very low for small mutation effects, and an enormously long time is required to reach this state. In our simulations (Fig. 3) time scale is measured in events, where an event is the fixation or loss of a newly arisen mutation (Welch & Waxman 2003) and the 10^6 substitutions simulated correspond to 10^5 years if time is measured by the unit of 1/v generations (with generations of one year). In such period of time, the distribution was maintained around the starting point and probably it takes a very long time to get to equilibrium for small step sizes (see Ohta & Tachida 1990 and Tachida 1991).

Thus, the relation between the mutation size and the creative effect of natural selection is not simple. According to our results the size of mutations in small populations has two principal effects (Fig. 3). Large mutation sizes make that the evolutionary trait state of populations move speedily to the mutation-selection-drift equilibrium distribution. Hence, for low dimensions (i.e., low complexity of the trait), larger mutation size bring near the trait toward a more adaptive state than the state followed by the recent environmental change. Thus, large mutations increase the serendipitous adaptations of the trait, i.e. increase the probability that the trait becomes adaptive by chance. Second, for higher dimensions (i.e., high complexity of the trait), larger mutations moves the trait state toward an equilibrium distribution with a worse mean state, and thus the serendipitous adaptations of the trait could decrease. Nevertheless, the dispersion of the distribution at the equilibrium state increases with the increasing of the mutation size, then, it is possible that the serendipitous adaptations increases with the mutation effect when the starting point of population is very low (high distance to the optimum). Theoretical work about the fixed drift load in the FGM has been centered on the fitness average of the fixed drift load but not on the fitness variance (e.g., Hartl & Taubes 1996, Poon & Otto 2000, Sella & Hirsh 2005, Sella 2009). Probably theoretical models on population extinction risk (Poon & Otto 2000, Whitlock 2000, Whitlock et al. 2003) could be affected if the fitness variance is considered. With respect to the mutation-selection-drift equilibrium state, when the complexity of a trait increases (i.e. for traits with a higher number of dimensions) the probability of its adaptation by chance decreases. This could be explained because the pressure to deleterious changes is higher for higher dimensions (Fisher 1930, Hartl & Taubes 1996). Thus the serendipitous adaptations only would be important for simple traits or for genes of low

pleiotropy -if dimensions are interpreted as the degree of pleiotropy of a gene (Gu 2007a, 2007b).

It is important to consider that substitution events are in an ensemble of populations. Thus, each distributions obtained is a distribution among an ensemble of populations, not the distribution of phenotypes within a single population. An assumption within this model is that at any one point in time, each population is genetically homogeneous (Hartl & Taubes 1998). Thus, while a trait of a population could suffer a process of high serendipitous adaptations, chance could be not beneficial for this trait in other population into the same species. Therefore, apparently local adaptation could be also the effect of serendipitous adaptation. On the other hand, if separated small populations experience a speciation process, as in the peripatric speciation (Mayr 1963, Gould 2002), serendipitous adaptations could be important. Molecular evolutionary models are based in the plausible fact that many speciation processes involve bottleneck, but authors only study the deleterious mutations fixed during speciation, and not consider the higher probability of fixation of advantageous mutations by chance during the bottleneck (e.g., Ohta 1993, Hughes 2007).

The first simulations in our study assumed an absolutely stable fixed optimum, but this scenario is not realistic (Orr 1998, 2005). Environments rarely remain constant, but fluctuate over time in a periodic fashion (seasonal changes) or in a much more unpredictable manner. Abiotic environmental stochasticity usually come first to mind (e.g. frost, fire, volcanic eruptions, asteroid collisions, and so on), but biotic environmental stochasticity is extremely frequent (Lenormand et al. 2008). This idea described as "biotic drift" (Turner & Mallet 1996) come from facts that ecological and demographic regimes of

parasites, predators, prey and even conspecifics can be particularly erratic (May 1976), specially with many species interacting (Hasting 2004). Abiotic and biotic environmental changes have been considered relevant to evolution, particularly fluctuations arising at long timescales (Lenormand et al. 2008). We model stochastic environment as random shift of the optimum trait at long time scale with a Gaussian distribution (Gu 2007). For large effective population sizes (N = 1000) distributions of fitness values ranged in fewer fitness values when increase both the variability and amplitude of environmental changes (Fig. 4). Thus, the superposition area between a drift-dominated and a selection-dominated process (Fig. 2) should increase for the increasing of variability and amplitude of changes. That is, the serendipitous adaptations of a trait must be higher in more variable environments.

Finally, when we studied the proportion of advantageous substitutions in a drift-dominated process we obtained a heuristic null model for the creative effect of natural selection. Because the fixation of advantageous mutations in a drift-dominated process is high (see above) the simple finding of advantageous substitutions does not guarantees that it was fixed by natural selection. In fact, as the expected proportion of advantageous/(advantageous+deleterious) substitutions is 50%, it must be found a high proportion of advantageous substitutions to guarantee statistically that an advantageous proportion cannot be explained by a stochastic process, higher when the number of substitutions is low (Fig. 5, Table 1). The null model presented into this study must be considered only as a preliminary heuristic method to test the creative effect of natural selection with empirical data. We think that in principle the method could be used to test the creative effect of natural selection in protein sequences. Nevertheless, because the number of dimensions affect the critical values (Table 1), to make a contrastable null hypothesis from the FGM it

would be necessary to know the dimensionality of the protein studied. Recently, it has been estimated the dimensionality of several proteins in the context of FGM (Gu 2007b), but such values are not robust yet. According to our knowledge, our heuristic null model is the first attempt to make the creative effect of natural selection measurable. It is possible that equivalent null models could be constructed to contrast empirical data under other theoretical framework than FGM. For example, using sequence proteins data, the Gillespie's "sequence space" model could be used (Gillespie 1984, 1991, Orr 2005). Recent developments integrating several levels of study, from phylogenetics to genetics are able to detect the specific nucleotide or amino acid substitution associated to the evolutionary transitions of adaptive traits (Golding & Dean 1998, Dean & Thornton 2007). Substitutions with an effect that tend to the derivate phenotype are considered single advantageous substitutions. Notably, the number of these advantageous substitutions usually is only one (Hughes 2007) or minor than a dozen (Hughes 2007, Dean & Thornton 2007, see Table 3 of Nei 2005 and Table 1 of Hoekstra & Coyne 2007), and in this range is very difficult to reject the null hypothesis (Table 1). A message of the null model is that a qualitative test to evaluate the creative effect of natural selection on these advantageous cumulative protein substitutions entails the assessment of the number of deleterious substitutions too. If the number of deleterious substitutions is close to the number of advantageous substitutions, the creative role of natural selection is strongly questionable, especially if the number of advantageous+deleterious substitutions is low. In the case of functional synthesis studies (Dean & Thornton 2007) deleterious substitutions could be estimated as substitutions which phenotypic effect tends against to the derivative phenotype. These results also reject the common assumption of the "two mutation threshold" of Huxley (1942) as, for example, with four or five advantageous mutations fixed the creative effect of natural selection only

could be guaranteed statistically if there is no deleterious mutation fixed (Table 1). However, the intuition that phenotypic changes reached by a low number of steps could be not attributable to natural selection is supported by the current study. The creative role of natural selection is a combinatory role (Simpson 1947, Mayr 1963, Gould 2002, Nanay 2004, Ayala 2007, see Appendix B). Thus, only if the evolutionary formation of a trait involves many advantageous changes (many adaptive mutations) natural selection could be considered creative. That is, only if the combination is not possible to obtain in a stochastic process the creative effect of natural selection could be plausible.

Our study goes against a long history of skepticism about the possibility to contrast chance with natural selection in the trait formation: "When one attempts to determine for a given trait whether it is the result of natural selection or of chance (the incidental byproduct of stochastic processes), one is faced by an epistemological dilemma. Almost any change in the course of evolution might have resulted by chance. Can one ever prove this? Probably never." (Mayr 1983). Our strategy is not trying to prove the hypothesis of trait formation by chance but trying to discard trait formation by chance taken it as the null hypothesis. The current molecular statistical tests of selection are not applicable for the low number of adaptive substitutions found in the functional synthesis studies (Hughes 2007). These tests, however only consider exclusively molecular information, with consequent loss information by not using the possible phenotypic effects of stochastic processes on the phenotype. We propose that taking this information account is possible attaining the level of resolution necessary to discard that stochastic processes (e.g. drift) are the responsible for the molecular pattern.

It is important to consider that our models depict the evolutionary process as a succession of fixations and neglect the effects of polymorphisms, and therefore provide only an approximation valid when the mutation rate is low ($\nu < 1/N$). Besides, we simulated the drift-dominated process with effective population sizes of ten individuals, which corresponds to a census population size of few hundred (Frankham 1995) that could represent extremely small populations even for higher organisms. Nevertheless, Whitlock (2000) maintains that populations have a critical population size below which they probably go to extinction by the genetic meltdown process (Lynch & Gabriel 1990, Poon & Otto 2000). Very approximate estimations predict that the critical effective population size is around a few hundred (Whitlock 2000, Whitlock et al. 2003), which could imply that populations in our simulations are in an extremely stochastic scenario that would carry it to extinction. Nevertheless, contrary to Whitlock (2000), Poon and Otto (2000) predicted that the effective population size does not decline below the number of dimensions of the organism (for a comparison between these models see Whitlock et al. 2003) and populations of a ten of individuals is an heuristic and usual extreme in population genetic models (e.g. is used in some simulations by Whitlock et al. 2003). Overall, our simulations could be considered conservative in the sense that the influence of natural selection is lower than the smallest population with long-term persistence. Moreover, other assumptions could be relevant for our results as the perfect symmetry of the fitness profiles and mutations sizes. In other context these assumptions have been relaxed for the FGM (Martin & Lenormand 2006, Waxman 2006) and it should be studied in the future.

Finally, we remark that our approach was proposed to differentiate the capacity of natural selection to form complex adaptive traits with the capacity of a stochastic

evolutionary process to produce traits with an equivalent fitness than those under natural selection. Nevertheless, this is not the only option. It has been affirmed that features that are complex or coordinated and that "appear to be adaptations" can be constructively created by random fixation of neutral variations (Stoltzfus 1999). Our approach differs from this viewpoint in that we understood an adaptation as a trait that achieve high fitness, and the null model is constructed on the scenario by which trait with high fitness is reached by random fixation of advantageous mutations, not by neutral mutations. Any study analyzing these approaches could be insightful for a better understanding of the origin of complex traits in evolution.

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Table 1. Critical values for proportion of advantageous substitutions specifying a neutral null hypothesis determining the reject zone for a level of significance of $\alpha = 0.05$ and $\alpha = 0.01$. Parameters used were $N = 10$, $r = 0.01$.

N° substitutions	Critical proportion (α=0.05,n=5)	Critical proportion (α=0.01,n=5)	Critical proportion (α=0.05,n=15)	Critical proportion (α=0.01,n=15)
1	-	-	0.99	0.99
2	-	-	0.99	0.99
3	-	-	0.99	0.99
4	0.99	-	0.99	0.99
5	0.79	-	0.79	0.99
6	0.82	0.99	0.82	0.99
7	0.84	0.84	0.84	0.84
8	0.74	0.86	0.74	0.86
9	0.77	0.88	0.77	0.88
10	0.79	0.89	0.69	0.79
15	0.72	0.79	0.72	0.81
20	0.69	0.74	0.64	0.74
25	0.63	0.71	0.63	0.71
30	0.62	0.69	0.62	0.69
35	0.62	0.67	0.62	0.67
40	0.61	0.66	0.61	0.66
45	0.61	0.66	0.59	0.66
50	0.59	0.65	0.59	0.65
55	0.59	0.64	0.59	0.64
60	0.59	0.64	0.59	0.62
65	0.59	0.62	0.57	0.62
70	0.57	0.62	0.57	0.62
75	0.58	0.62	0.58	0.62
80	0.58	0.61	0.56	0.6
85	0.58	0.6	0.57	0.6
90	0.57	0.6	0.57	0.6
95	0.57	0.6	0.56	0.6

Table 2. Terms for genetic phenomena associated to different evolutionary forces and different kinds of mutations. We call "serendipitous adaptations" to the fixation of advantageous mutations in a stochastic scenario.

Evolutionary force	*Kind of mutation*		
	Deleterious (s<0)	**Neutral (s=0)**	**Advantageous (s>0)**
Loss by selection	*Negative selection*	Background selection (by link)	Background selection (by link)
Fixation by selection	Selective sweep (by link)	Selective sweep (by link)	*Positive selection*
Fixation by drift	Nearly-neutral evolution	Neutral evolution	Serendipitous adaptation

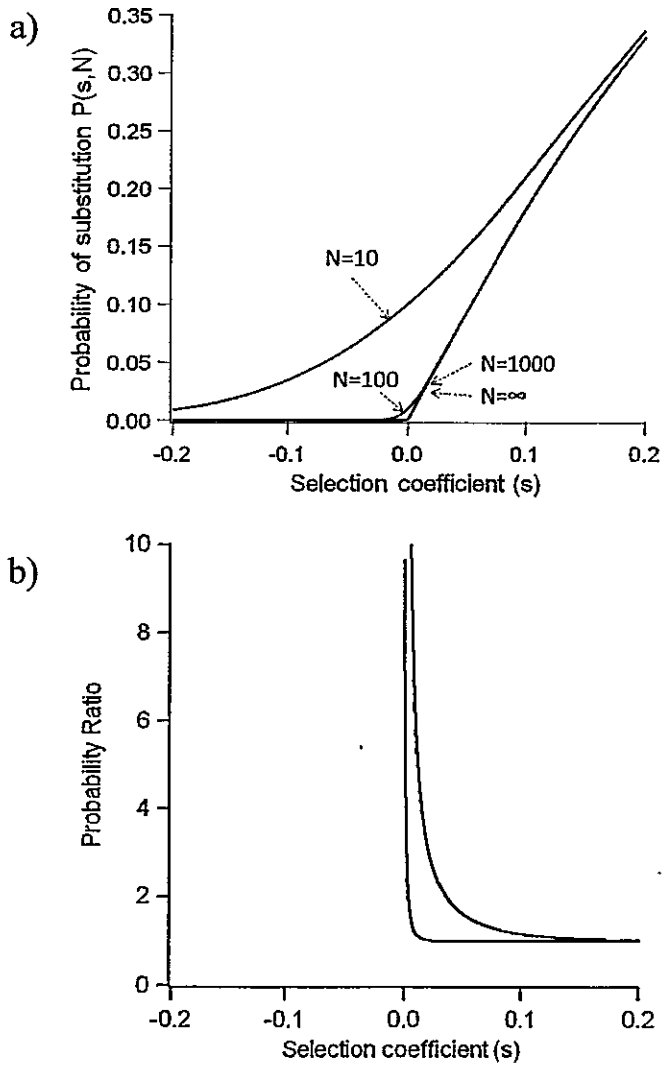

Figure 1. a) Probability of substitutions according to selection coefficients with population sizes $N = 10$, 100, 1000 and infinite. Curves for $N = 1000$ and $N = \infty$ are indistinguishable at that scale. b) Ratio between the probabilities of fixations of advantageous mutations for different population sizes. Right curve plots $P(s, N = 10) / P(s, N = 1000)$ and left curve plots $P(s, N = 100) / P(s, N = 1000)$.

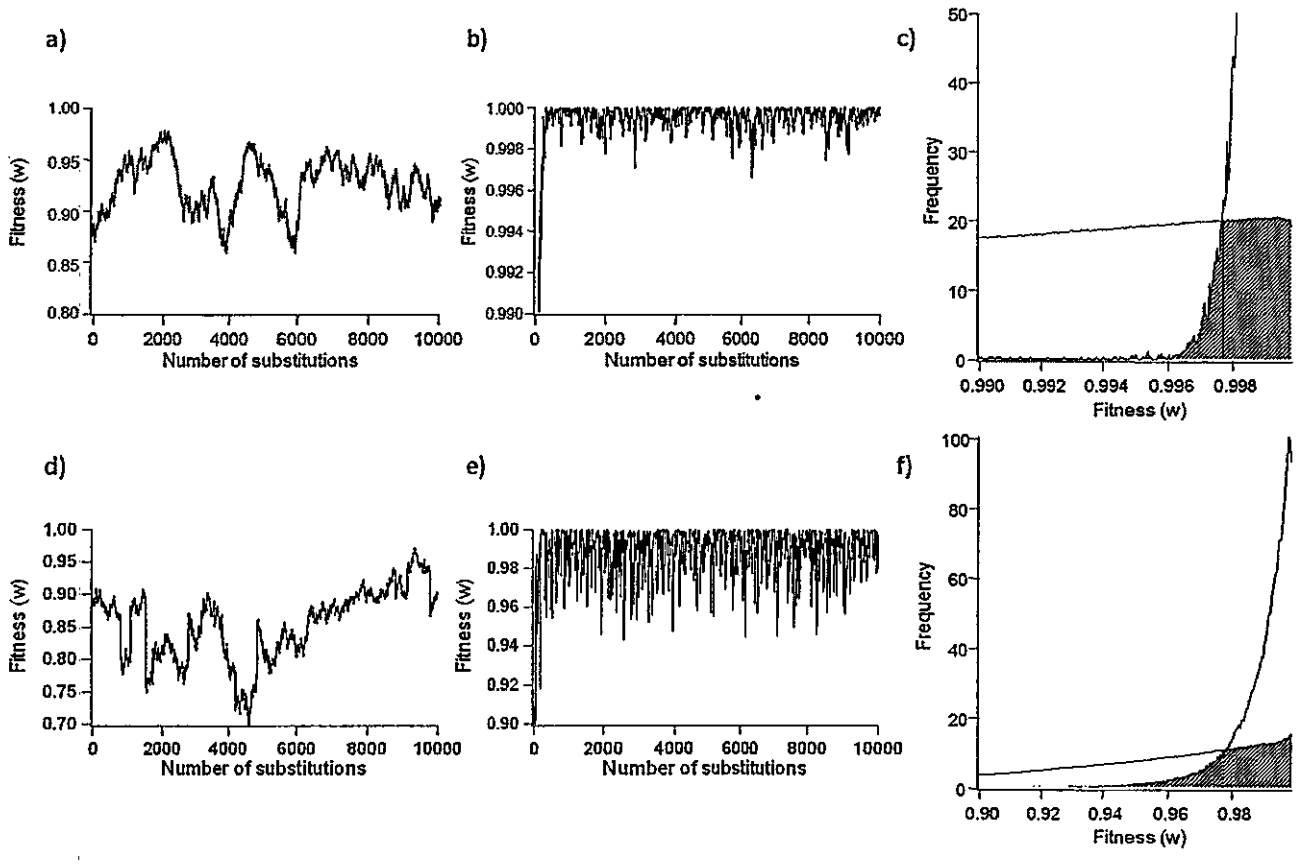

Figure 2. Comparison between fitness values reached in stochastic and selective regimes for $n=2$ dimensions. a) and b) represent the dynamic of fitness values for populations with $N=10$ and $N=1000$, respectively, in a stable environment. In c) is plotted the normalized fitness distributions for many walks like a) and b). The superposition area between distribution curves was 5.5%. d), e) and f) are the equivalent plots within variable environments. Parameters used for environmental variability were $\sigma_a=0.15$ and $\tau=1\cdot10^4$ in mutational time units. The superposition area between distribution curves was 37.3%. Distributions were generated with 10^5 substitutions.

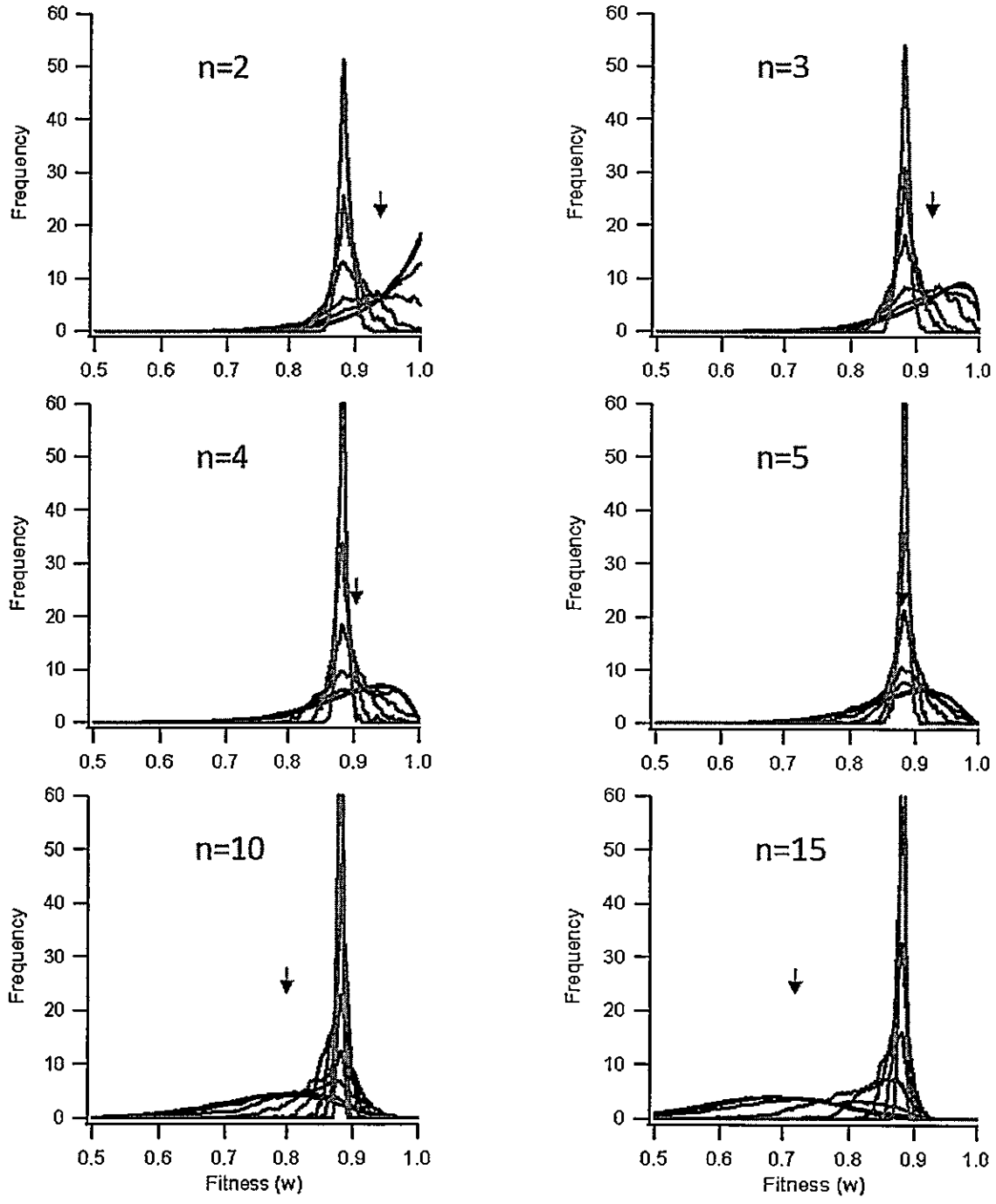

Figure 3. Normalized distributions of fitness values. From brighter to darker curves represent mutational size of $r = 0.02, 0.01, 0.005, 0.0025, 0.00125, 0.000625, 0.0003125, 0.00015625$, respectively. Different dimensions are indicated in each plot: $n = 2, 3, 4, 5, 10$

and 15. Theoretical values to mean expected fitness are indicated by an arrow point according to the formula $W_{eq} = (1-(2N-1)^{-1})^{n/2}$ (Tenaillon et al. 2007). Each curve corresponds to 10^6 substitutions.

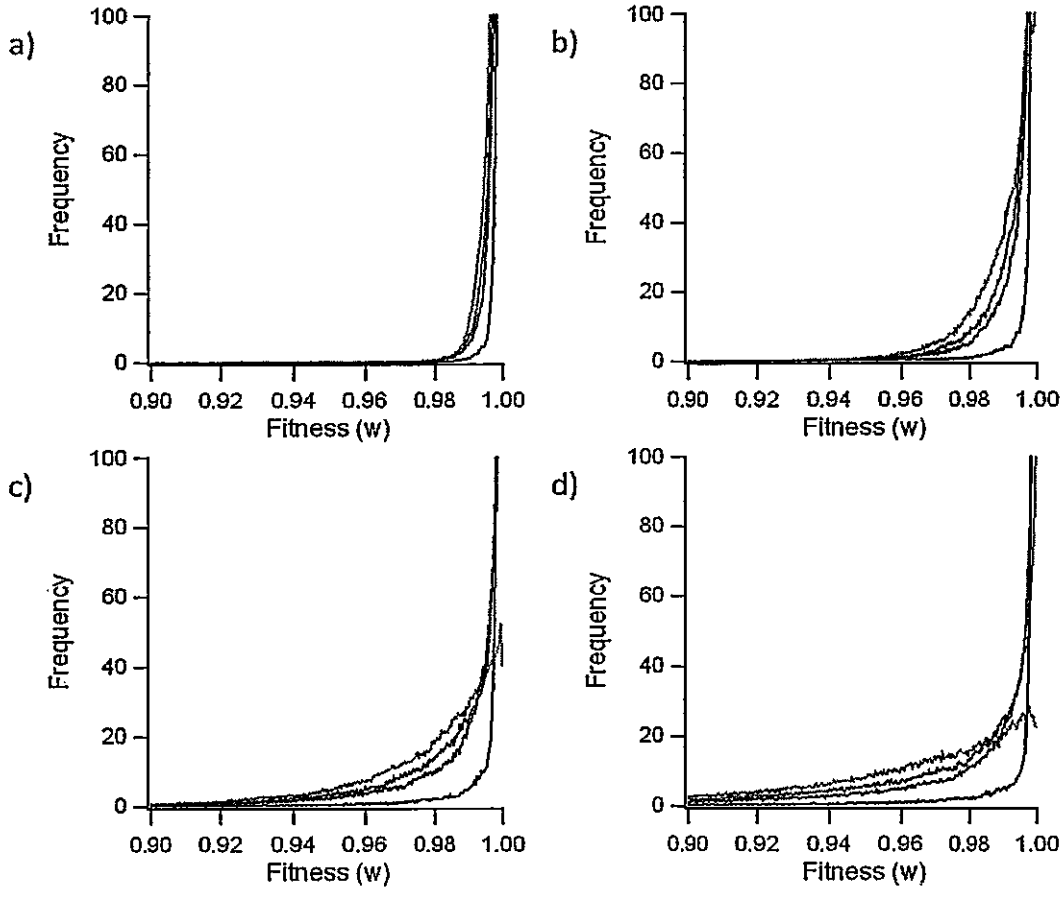

Figure 4. Normalized distributions of fitness values incorporating variable environments. General parameters used were $N = 1000$, $n = 2$, $r = 0.005$. Standard deviations of optimum shifts amplitudes corresponds to a) $\sigma_a = 0.07$. b) $\sigma_a = 0.15$. c) $\sigma_a = 0.2$. d) $\sigma_a = 0.3$. Each plot presents results for different temporal variability: from brighter to darker is expected one environmental change each $\tau = 1 \cdot 10^4$, $5 \cdot 10^4$, $1 \cdot 10^5$, $1 \cdot 10^6$ mutational times, respectively. Each curve corresponds to 250.000 substitutions.

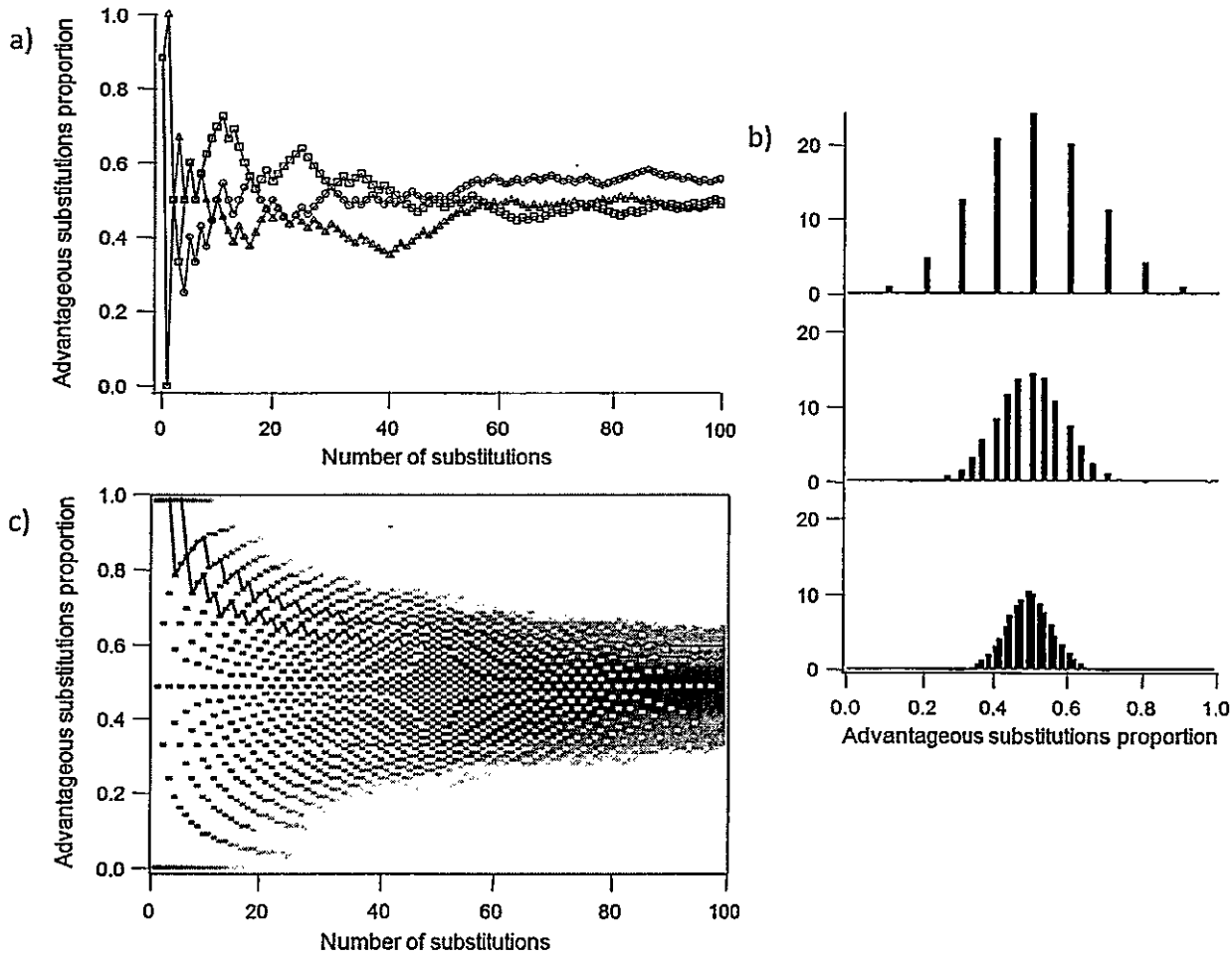

Figure 5. Advantageous substitutions proportion, (advantageous)/(advantageous +deleterious), in a drift-dominated evolutionary process with $n=5$, $N=10$, $r=0.001$. a) Advantageous substitutions proportion for three trials of evolutionary walks. b) Normalized distribution of advantageous substitutions proportion for 10, 30 and 65 substitutions (from top to down). c) Normalized distribution of advantageous substitutions proportion for a range of number of substitutions. Grid scale represent the frequency of different proportions (darker correspond to more frequent proportions). Continue lines indicate critical values to reject the null hypothesis for a significance level of $\alpha=0.05$ (lower line) and $\alpha=0.01$ (upper line). b) and c) come from simulations with 100.000 walks.

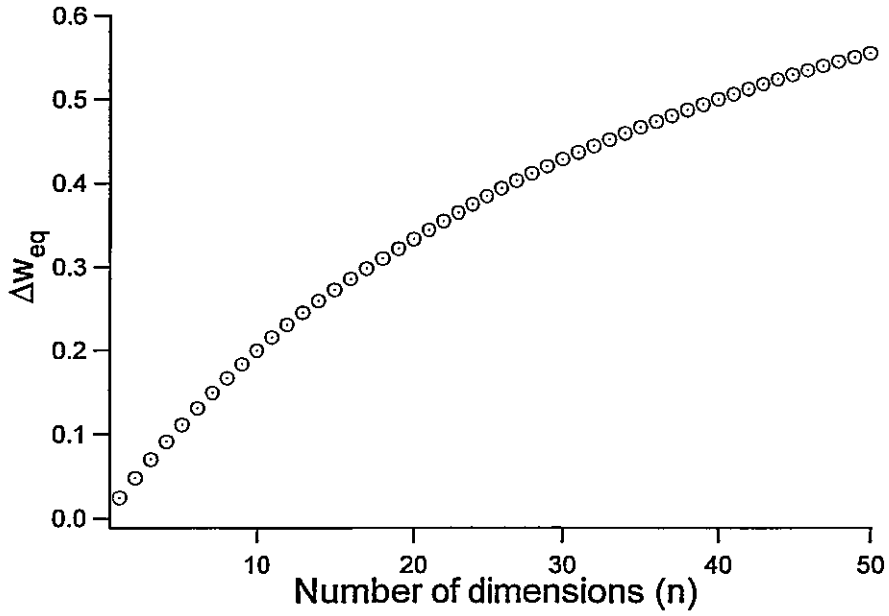

Figure 6. Theoretical difference between equilibrium mean fitness expected for populations with $N = 1000$ and $N = 10$ for different number of dimensions, according to the formula $\Delta w_{eq} = [(1-(2000-1)^{-1})^{n/2}] - [(1-(20-1)^{-1})^{n/2}]$.

CAPÍTULO 3

EL ROL DE LA PLEIOTROPÍA EN LA EVOLUCIÓN MOLECULAR

The rate of molecular evolution and the phenotypic complexity of mutational effects

ABSTRACT: How the phenotypic effects of genetic mutations influence the molecular evolution is a not well-understood theme. Neutral and nearly-neutral theories of molecular evolution predict a negative relationship between the evolutionary rate and the functional importance of a protein, nevertheless empirical endeavours seeking relationships between evolutionary rate and phenotypic impact of proteins has not conveyed conclusive results. Particularly, previous studies have not found the expected negative correlation between evolutionary rate and gene pleiotropy. Here, we study the effect of the mutation size and pleiotropy of genes on the evolutionary rate in a geometrical model, where gene pleiotropy is characterized by n molecular phenotypes that affect organismal fitness. We found that for a nearly neutral process is predicted a negative relationship between evolutionary rate and mutation size but pleiotropy does not affect the evolutionary rate. Further, we found that a selection model, where most of substitutions are advantageous mutations fixed by natural selection in a random fluctuating environment, also predicts the negative relationship between evolutionary rate and mutation size and that gene pleiotropy increases the evolutionary rate. These findings could explain part of the disagreement between empirical data and traditional expectations.

KEY WORDS: molecular evolution, gene pleiotropy, cost of complexity, principle of molecular evolution, Fisher's geometrical model

INTRODUCTION

The evolutionary rate of amino acid substitutions in a gene depends on the fitness consequences of such substitutions. A pattern early accepted in molecular evolutionary biology is that functionally less important molecules or parts of a molecule evolve faster than more important ones, which was considered a principle of molecular evolution (Kimura & Ohta 1974). Early examples of this principle in proteins was centered on the structural constraints of a few proteins (Dickerson 1971), but recently there have been relevant efforts aimed to obtain systematic patterns from a high number of genes and for different measures of biological role (Pal et al. 2001; Fraser et al. 2002, Wall et al. 2005, Salathé et al. 2006). The functional importance of a protein has been measured as dispensability (Wall et al. 2005), expression level (Pal et al. 2001), multifunctionality (Salathé et al. 2006), and connectivity (Fraser et al. 2002), but the results of these approaches have not been conclusive (Camps et al. 2007). In this respect, it is particularly surprising the apparent lack of correlation between pleiotropy of a given protein and its rate of evolution, measured as the ratio of nonsynonymous to synonymous substitutions (dN/ds) (Camps et al. 2007). For example, Salathé et al. (2006) studied the relation between the rate of molecular evolution and the pleiotropy of genes in *Saccharomyces cerevisiae*. Pleiotropy was measured as the number of biological processes in which a gene is involved, according to the Gene Ontology (The Gene Ontology Consortium 2002) project. The authors expected to find a negative correlation between evolutionary rate and pleiotropy (Otto 2004, Pal et al. 2006) but they only found a very weak correlation and concluded that their results do not support the notion that multifunctionality limits a gene's rate of evolution. Similarly using data from a study that quantifies pleiotropy based on phenotypic

effects on growth in different environments (Dudley et al. 2005), Salathé et al. (2006) concluded again that pleiotropy has a limited impact on a gene's rate of evolution.

According to Camps et al. (2007) these results and others similar (e.g., Jordan et al. 2003, Hahn et al. 2004) imply strong inconsistencies with the neutral and nearly-neutral theories of molecular evolution. In fact, the principle has been considered one of the strongest evidence for the neutral and nearly-neutral theories of molecular evolution (Kimura & Ohta 1974, Kimura 1983, Ohta & Gillespie 1996, Hughes 2007). If effectively advantageous mutations ($s > 1/N$) are very rare, only mutations with neutral or nearly-neutral effect could be fixed. Thus, important proteins would evolve slowly because many mutations would have a severe and detrimental impact on fitness ($s < -1/N$) and few mutations would be neutral and nearly-neutral. On the contrary, less important proteins would evolve faster because more mutations would be neutral or nearly neutral. Nevertheless, in the current study we suggest that the principle is not incompatible with a selection model of molecular evolution (i.e., a model where the majority of substitutions are fixed by positive natural selection, Gillespie 1994b). Indeed, based on his geometrical model, Fisher (1930) argued that positive selection tends to favor the less important changes and disfavors the important ones because larger mutations have a lower probability to be advantageous, thus predicting the same behavior than the one adjudicated to the neutral theories (Clarke 1971, 2001). Kimura and Ohta (1974) discarded that the principle can be explained by positive natural selection because smaller advantageous mutations are less probable to fix by selection (see also Jukes & King 1971). Then selection disfavors smaller and favors larger advantageous mutations. This argument was incorporated by Kimura (1983) into the Fisher's geometrical model (FGM) reaching to the result that the

distribution of advantageous mutations fixed by natural selection has to be a peak in an intermediate mutation effect size, but Orr (1998) later showed that the argument of Kimura (1983) was misleading because he did not consider the complete bout of adaptation experimented by a population, but only the first step. Orr (1998, 1999) estimated the distribution of sizes of factors fixed in a complete bout of adaptation and found that it is roughly exponential. Although Orr's studies do not target the rate of substitutions, the exponential distribution founded suggests that a model considering adaptive processes in the FGM could predict the principle of molecular evolution too.

Here we studied a model of evolution based on the FGM to analyze the principle of molecular evolution. The FGM is particularly useful to study the evolutionary relevance of the complexity of mutation effects. A mutation is represented as a vector in an n-dimensional space, where the number of dimensions represents the number of traits affected by the mutation and the magnitude of the vector represents the size of the mutation effect. Gene pleiotropy can be understood as the capacity of a gene to affect multiple phenotypic characters and modeled as the number of dimensions in the Fisher's multidimensional space (Gu 2007a, 2007b). Thus, in the FGM is possible differentiate between the size of a mutation effect (the Cartesian magnitude of vectors) and gene pleiotropy (the number of dimensions of the multidimensional space).

We showed that there is no negative relationship between pleiotropy and evolutionary rate when the effect of mutation size is controlled. Our simulations also revealed that the negative association between mutation size and evolutionary rate is predicted both by a nearly-neutral and by a selection model with random environmental change. Furthermore,

we suggest that a selection model could explain the weak empirical correlation observed between pleiotropy and evolutionary rate.

METHODS

Assumptions

Gene pleiotropy is characterized by n distinct components that affect organismal fitness, henceforth "molecular phenotypes" (Gu 2007a, 2007b, see also Hartl et al. 1985). The possible effects of mutations on the gene are represented as a multidimensional space of orthogonal phenotypic components. A strong assumption of the model is that a protein has an optimum molecular phenotype independently of the influence of other genes. Besides, this optimum can be shifted either by environmental changes or by internal physiological perturbations (Gu 2007b). When a mutation is fixed the phenotype of individuals in the population is modified to a new value, thus, the subsequent mutations start from the new phenotypic state.

Usually, the evolutionary process in the FGM is modeled in a bout of adaptation after a recent environmental shift of the optimum (Orr 1998, 1999, 2000, Welch & Waxman 2003), but the unavoidable fate of an adaptive bout is to reach to a balanced steady state where the molecular evolution does not stop (Hartl & Taubes 1996). Properties of the balanced steady state have been studied maintaining a fixed optimum (Hartl & Taubes 1998, Poon & Otto 2000, Sella & Hirsh 2005, Tenaillon et al. 2007, Sella 2009). In our simulations we modeled a temporally fluctuating random optimum shift, thus the

evolutionary process is an alternation between adaptive bouts and balanced steady states that will be determined by the variability of the environment changes.

We study the molecular evolution as the ratio between the rate of substitutions and the rate of mutations (k/u), which it usually measured by dN/dS empirical data, under the assumption that synonymous substitutions are almost neutral. In order to differentiate between the influence of mutation size and pleiotropy in the substitution rate of a gene we utilized a top-down approach to the random vectors generation (Poon & Otto 2000). That is, it is specified the distribution of total mutational length and left unspecified the component distributions along each axis. Thus, a change in the number of dimensions does not affect the total length of mutation effects. The distribution of magnitudes used is uniform, implying that in each axis the distribution of effects is leptokurtic, in correspondence with the empirical evidence (see Keightley 1994, Lynch et al. 1999).

We modeled asexual populations under weak mutation ($Nu \ll 1$), thus the evolutionary process is depicted as a succession of fixations and neglects the effects of polymorphisms.

Simulations

Simulations were made by Monte Carlo methods where random vectors are generated into a n-dimensional space of phase, with a uniform distribution of vector magnitudes (from 0 to r) (following Kimura 1983, Orr 1998). These changes can be fixed according to the

probability function $p(N,s)=\frac{1-e^{-2s}}{1-e^{-2Ns}}$, where N is the effective population size and s the selection coefficient of the mutation (Crow & Kimura 1970).

Fitness values were determined by the Gaussian function $w(z)=e^{-\frac{z^2}{2}}$, where z is the distance to the optimum point. Selection coefficients are defined as $s=\frac{w_{mut}-w^+}{w^+}$, where w_{mut} is the fitness of the mutant and w^+ is the fitness of the wild-type. We obtained the ratio substitution rate / mutation rate for different conditions: degree of pleiotropy (number of dimensions), size of mutations (vector lenght), population size, amplitude and variability of optimum shifts due to environmental change.

Random environmental shifts were simulated as a Poisson process such that $\nu \sim f(\nu,\lambda)=\frac{e^{-\lambda}\lambda^{\nu}}{\nu!}$, where ν and λ are the number and the expected number of changes in a time interval, respectively. Time intervals between consecutive changes (t) follow an exponential distribution $t \sim f(t,\tau)=e^{-\frac{1}{\tau}t}$, where $\tau=1/\lambda$ is the expected time between environmental changes (pseudo-random numbers were obtained using *expnoise* function available in IgorPro, WaveMetrics, Lake Oswego, OR). In order to compare results between different population sizes in variable environments we set that $\tau \propto N$. The amplitudes of environmental changes were calculated as $a(z_1...,z_n)=\frac{\sqrt{{z_1}^2+...+{z_n}^2}}{\sqrt{n}}$ where z_i are the coordinates of the new optimum which were chosen from a Gaussian distribution centered in the zero point of the Cartesian n-dimensional space, $z_i \sim f(\sigma_a)=e^{-\frac{z_i^2}{2{\sigma_a}^2}}$, where

σ_a represents the standard deviation of amplitudes of environmental changes (we used *gnoise* function available in IgorPro, WaveMetrics, Lake Oswego, OR).

Some simulations differentiate between affectively advantageous, effectively neutral, and effectively deleterious substitutions. Effectively advantageous substitutions are defined as substitutions that accomplish $s > 1/N$, i.e. advantageous substitutions fixed by positive selection; effectively neutral substitutions are defined as $|s| < 1/N$, i.e. substitutions fixed mainly by random drift; and effectively deleterious substitutions are defined as $s < -1/N$, i.e. deleterious substitutions fixed by drift in spite to its strong negative selection against them.

RESULTS

In the Figure 1 we show a trial of the substitution process used for simulations with random environmental variability. After an optimum shift due to environmental change, the population suffers a burst of adaptive substitutions until reaching a balanced steady state. In the balanced steady state, the population remains around a sub-optimum equilibrium fitness that is lower for higher number of dimensions and where a fluctuating substitution process occurs (Fig. 1). The time to reach to the steady state is higher for more pleiotropic genes (darker curve in Fig. 1) than less pleiotropic ones (brighter curve in Fig. 1).

We found a negative relationship between the effects of the size of mutations and the average selection coefficient of mutations, even for strongly fluctuating environments (Fig. 2). Consequently, the distribution of mutations selection coefficients is skewed toward negative values even with strong environmental change, where the distribution of

substitution selection coefficients is skewed to positive values (Fig. 3). In all of conditions studied there is a negative relationship between evolutionary rate and size of mutation effects (Fig. 4). Higher population sizes present lower evolutionary rate than lower population sizes when the amplitude of optimum shifts is null or low (Fig. 4a-c). This pattern is attenuated for higher shift amplitudes (Fig. 4d,e).

For lower size populations the evolutionary rate is insensitive to environmental variability, but populations with higher size increase considerably its evolutionary rate with the amplitude and variability of environmental changes (see Fig. 4 and Fig. 5a). The increasing of evolutionary rate is higher for more pleiotropic proteins (Fig. 4 and Fig. 5). This increasing is due to the effectively advantageous substitutions, while the effectively neutral and deleterious substitutions are insensitive to the degree of gene pleiotropy (Fig. 5a). When the optimum is maintained constant (stable environment) the degree of pleiotropy does not affect the evolutionary rate (Fig. 5a).

The rate of effectively advantageous substitutions becomes higher than the rate of effectively neutral mutations since a critical number of dimensions ($n_c = 8$ for $\tau = 10^4$ and $n_c = 35$ for $\tau = 5 \cdot 10^5$ in Fig. 5a), and this effect is higher for more variable environments (Fig. 5). This implies that more pleiotropic proteins in variable environments (with a mean time between shits of 100, 500 and 1000 generation time in Fig. 5) fall under a selection model, where the majority of substitutions are advantageous mutations fixed by natural selection. The increasing of effectively advantageous substitutions rate with the number of dimensions is higher for lower mutation sizes (Fig. 5b).

DISCUSSION

The phenotypic complexity of the effect of a protein mutation depends on an intricate array of factors and interactions among factors, including the structural constraint on protein folding, stability and activity (DePristo et al. 2005), gene regulatory and protein interaction networks (Ohta 2007) and various epigenetic, developmental and environmental aspects. In turn, the evolutionary fate of mutations depends on these complex phenotypic effects (Ohta 2007).

The current information available in data banks have made possible the searching for correlations between evolutionary rate and some indicators of the complexity of protein mutation effects. Nevertheless, theoretical predictions relating molecular evolution and phenotypic change are scarce. Particularly, pleiotropy has been largely ignored in traditional population genetics models (but see Griswold & Whitlock 2003, Otto 2004). In our model we found that the traditional prediction stating that more important proteins evolve at a slower rate than less important proteins is expected under a wide range of conditions, if "important" is interpreted as the overall size of the phenotypic effect of protein mutations (Fig. 4). Interestingly, this pattern is predicted both for a nearly-neutral condition where most of substitutions are fixed by random drift and for a selective condition in a random fluctuating environment where most of substitutions are fixed by positive natural selection. This is in strong opposition to the FGM-based prediction of Kimura (1983) of a "maximum evolutionary rate occurring for an intermediate mutational effect" (p. 155), reflecting the deficiencies of Kimura's assumptions previously treated by Orr (1998) in the context of the distribution of mutation size. Thus, the principle of molecular evolution could be not necessarily understood as evidence for the neutral or

nearly neutral theory as it has been traditionally maintained (Kimura & Ohta 1974, Kimura 1983, Ohta & Gillespie 1996). This occurs by way of two effects: first, because for any fixed distance to the optimum an increase in size strongly increases the number of deleterious mutations, as was predicted by Fisher (1930). Second, although advantageous mutations of higher sizes increase and they are more probable to be fixed by natural selection, the fixation of large substitutions occurs for a low proportion of mutations and for a short time period. Indeed, when the protein state progressively approximate to the optimum, the same mutations that would have been advantageous at the beginning becomes deleterious with time (which explain the exponential distribution founded by Orr (1998)). On the other hand, in absence of environmental change, the population remains in a dynamic balanced steady state. This state is commonly understood as a nearly-neutral evolutionary process (Hartl & Taubes 1996, Sella & Hirsh 2005, Sella 2009) and it is in some aspects similar to the house-of-cards nearly neutral model (Ohta & Tachida 1990, Tachida 1991, 1996). Under this condition the negative relationship between evolutionary rate and size of mutation effect is predicted too but, at variance with selection models, pleiotropy does not affect the evolutionary rate (Fig. 5a).

On the other hand, we found that gene pleiotropy, that is the number of orthogonal molecular phenotypes affected by the mutations on a protein, does not affect the rate of substitutions in a nearly-neutral, environmentally stable condition (Fig. 4a,b and Fig. 5a). This could explain the very weak correlation found between evolutionary rate and pleiotropy in empirical studies (Jordan et al. 2003, Hahn et al. 2004, Salathé et al. 2006, Camps et al. 2007). This result contrasts with the fact that in the FGM the probability that a mutation of a given size be advantageous decreases with the number of dimensions, and the

probability to be deleterious increases (Fisher 1930, Orr 1998). Interestingly, in the balanced steady state, this fact only affects the mean equilibrium fitness (that is lower for higher number of dimensions), but when the equilibrium is reached the rate of substitutions is not affected by the number of dimensions (gen pleiotropy). This is explainable because the lower equilibrium fitness for higher dimensions is farer to the optimum, and thus the number of advantageous mutations increase (Fisher 1930, Orr 1998). Thus the effect of the dimensions is compensated by the effect of the distance to the optimum.

Nevertheless, in our model we differentiate the effect of pleiotropy and the size of effects such that gene pleiotropy is not correlated with the size of mutation effects. It is likely that this is not the case in nature and it is to be expected that the higher is gene pleiotropy the higher is the overall phenotypic effect of gene mutations. For example, Wall et al. (2005) found a negative (although weak) correlation between dispensability of proteins (measured as the growth rate of a strain in which this gene was knocked out) and the number of biological processes in which they are involved in *S. cerevisiae* (Salathé et al. 2007). This relation between pleiotropy and fitness effects should imply an expected negative correlation between pleiotropy and evolutionary rate. The lack of empirical support for this prediction could be explained in our model when environmental variability is incorporated. Our results showed that substitution rate increase with gene pleiotropy in variable environments (Fig. 5). This could explain an attenuation of the negative correlation between evolutionary rate and gene pleiotropy. Thus, although mutations in more pleiotropic proteins tend to generate a phenotypic effect of higher size, its pleiotropic nature tends to increase its evolutionary rate above the expected rate, according to its effect size. This effect should be stronger for lower mutation size (Fig. 5b). These effects in the model

are due to a high rate of advantageous substitutions, a pattern observed in several empirical studies (see e.g., Fay et al. 2002, Bierne et al. 2004, Eyre-Walker 2006, Bachtrog 2008).

Our results about the influence of gene pleiotropy in molecular evolution could be related with the "cost of complexity" developed also in the FGM framework (Orr 2000, Welch and Waxman 2003). From a geometric point of view, the results about the substitution rate of more pleiotropic proteins could be interpreted as results about the genomic substitution rate of more complex organisms (i.e., organisms with a higher number of traits under mutational change and selection). Thus, more complex organisms should suffer a higher genomic rate of substitutions (net and strictly advantageous) than less complex organisms. This result is coherent with the cost of complexity, one of the most interesting results obtained from the FGM (Orr 2005). Orr (2000) has demonstrated that as the number of characters (i.e, complexity) increases, there is a significant reduction (cost) in the rate of adaptation. Welch and Waxman (2003) showed that Orr's finding is robust to many modifications of the model's assumptions. Indeed, more pleiotropic genes take longer time to reach to the steady state (Fig. 1). But an apparent paradox entails that the lower rate of adaptation in more complex organisms it has made expecting that "beneficial mutations should be less frequent in complex organisms" (Martin & Lenormand 2006b, similarly Pal et al. 2006). Contrarily, our simulations showed that the rate of advantageous mutations increases with complexity. However, this is not inconsistent with the cost of complexity but a consequence of it. More complex organisms have a lower rate of adaptation due in part because favorable mutations of a given size travel shorter distances to the optimum (Orr 2000, Welch & Waxman 2003). Thus, the increased evolutionary rate of advantageous substitutions for higher complexity is due to the higher number of substitutions necessary to

travel the same distance toward the optimum. Then more pleiotropic proteins spend more time in adaptive processes (with a higher Maynard Smith's "lag load", see Orr 2000) than less pleiotropic proteins, accumulating a higher number of small size advantageous substitutions. This becomes intuitive in the Figure 1. Consequently the increase of strictly advantageous substitution rate with the number of dimensions is stronger for lower mutation sizes (Fig. 5b).

Griswold and Whitlock (2003) found a similar behavior of the Orr's cost of complexity due to pleiotropic deleterious effects of mutations increase with the number of dimensions. They found that the average scaled size of a fixed effect per character decreases as the degree of phenotypic pleiotropy increases. They argue that this result is similar to Orr's (2000) result, and that it is not due to an artifact of the possibility that pleiotropy influences the average magnitude of a random mutation. Recently, Gu (2007a, 2007b) found a strong negative relationship between evolutionary rate and gene pleiotropy. Nevertheless, Gu's finding is due to his bottom-up approach to the random vectors generation (Poon & Otto 2000, Appendix 2 of Orr 2000). In this approach, the distribution and magnitude of mutation effects is specified for each axis and the total length of mutation is left unspecified. Thus, under the bottom-up approach, the magnitude of vectors increases as the number of dimensions increases (Appendix 2 of Orr 2000). In Gu's model, more pleiotropy decreases the evolutionary rate simply because pleiotropy increases the size of mutations. Thus, it does not enable studying the evolutionary consequences of gene pleiotropy with independence of its impact on the size of its mutational effects (other criticisms of the approach used by Gu (2007a, 2007b) can be found in the Appendix 2 of Orr (2000)).

The status of the principle of molecular evolution is well established for non-synonymous vs. synonymous or intronic substitutions ("parts of a molecule" *sensu* Kimura & Ohta 1974) in the same loci, and it is a well support of the neutral model for the evolution of synonymous nucleotide sites (Hughes 2007). However, the applicability of the principle to protein comparisons has been questioned (Camps et al. 2007). Part of the discrepancies between empirical studies and the traditional expectation could be explained because the measures of the biological role or functional centrality of a protein (connectivity, dispensability, multifunctionality, and essentiality) could be related to the number of molecular phenotypes affected by the protein. For example, connectivity in a protein network could be related to the pleiotropy of proteins (Camps et al. 2007). Therefore, more connected proteins could have a higher substitution rate than the expected rate according to their impact on fitness, weakening the correlation between substitution rate and connectivity. The empirical difficulty to account for the principle of molecular evolution in proteins could be explained in part because the pleiotropy of the effect and the size of the effect, contrarily to our model, are not separated in real systems. In our model they were separately treated in theoretical terms to predict their different evolutionary effects. Other consideration to take account for the interpretation of our model is that the number of orthogonal dimensions affected by a protein is not equivalent to the total number of traits affected by the protein if the effects on traits are correlated (e.g., if, for example, the increasing of a trait is not possible without some sacrifice in other trait). When there are correlations between traits the number of independent axes in the FGM decreases (Rice 1990, Martin & Lenormand 2006a, 2008). In fact, the number of dimensions could be fractional (Rice 1990). Let m the total number of correlated traits and n the number of orthogonal traits. When there are no or little correlation between traits, $m = n$, but if there

are correlation between traits $n < m$, and there are $m - n$ traits that can be expressed as linear combinations of the n distinct traits of the system (Martin & Lenormand 2008).

Our results also could be related to other line of evidence about the genetic basis of adaptation. It has been highlighted that tests of genetic adaptations have revealed structural genes with high number of mutations fixed by positive selection, while cis-regulatory (non-coding) regions show lower evidence of such signal (Hoekstra & Coyne 2007). This fact is used by Hoekstra and Coyne (2007) as evidence against the evolutionary developmental biology ("evo-devo") claim that adaptive mutations affecting morphology are more likely to occur in the *cis*-regulatory regions than in the protein-coding regions of genes (see King & Wilson 1975, Carroll 2000, 2005a, 2005b). The strongest evo-devo argument in favor to the preponderance of *cis*-regulatory elements is that mutations in these regions are largely free from pleiotropic (and thus, probably deleterious) effects (Stern 2000, see also Hoekstra & Coyne 2007). Thus, our results suggest that, whether our model with variability is applied to regulatory regions too, it is expectable that regulatory regions show fewer rates of mutations fixed by positive selection because they have lower pleiotropic effects, and not because they are less important in morphological evolution. Thus, it is possible that major adaptive shifts took place by molecular changes without an accelerated protein or DNA evolution (see also King & Wilson 1975).

It is important to take into account that our model makes some assumptions that could influence the results and that could be biologically unrealistic. Particularly we had followed the usual Gaussian fitness function and one peak assumptions, which could be considered unrealistic assumptions especially for strong environmental changes (Martin & Lenormand 2006b). Yet a number of evidences coming from experimental evolution,

distribution of mutation fitness, and stabilizing selection, indicate that this assumptions may not be quantitatively as unrealistic as previously suggested (reviewed in Martin & Lenormand 2006b). We also assumed symmetry of the mutation directions on a given protein. This assumption rests on the presence of compensatory mutations within the same molecule, which seems reasonable due to the current evidence of intragenic compensatory and suppressor mutations (Poon & Otto 2000, Whitlock et al. 2003, Poon et al. 2005, Pal et al. 2006), where the overall majority of compensatory mutations seems to be intragenic (Poon et al. 2005). Fitness equivalence between traits (symmetrical geometry of the model) is also a strong assumption in our model. Whitlock et al. (2003) simulations showed that some parameters, particularly the fixed drift load in the steady state, are insensible to the relaxation of the hypersphere by a hyperellipse. Nevertheless, to our knowledge no study has explored the effect of different degrees of environmental variability or amplitudes of optimum change for the different traits. These changes in the model should be explored.

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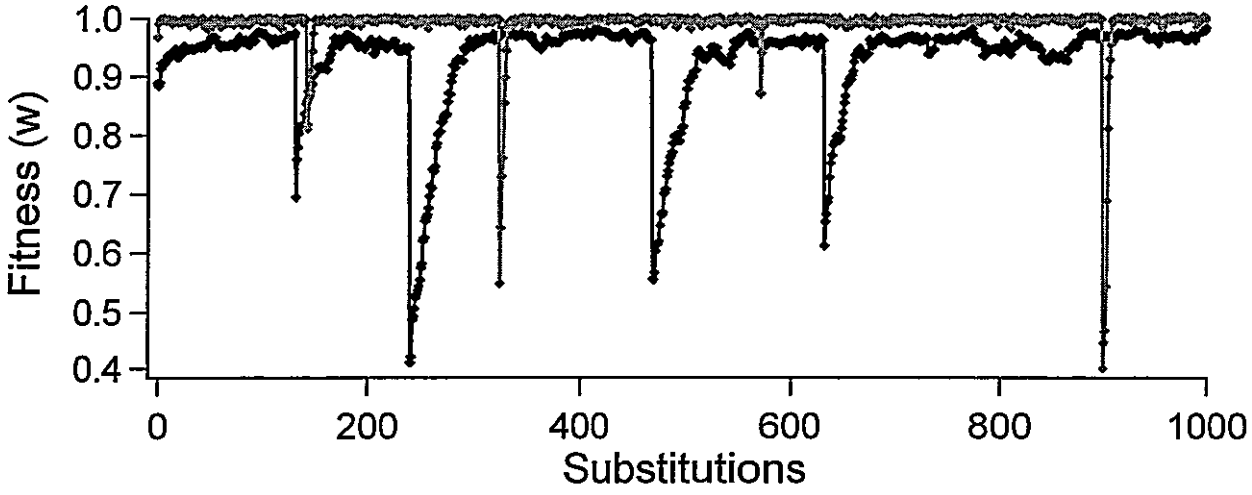


Figure 1. Sequence of substitutions in a random fluctuating environment for different number of dimensions. Brighter line corresponds to $n=3$ ($\tau=10^5$). Darker line corresponds to $n=30$ ($\tau=3\cdot10^5$). General parameters used were population size $N=200$, average mutation size $r/2=0.3$ and environmental amplitude $\sigma_a=0.85$.

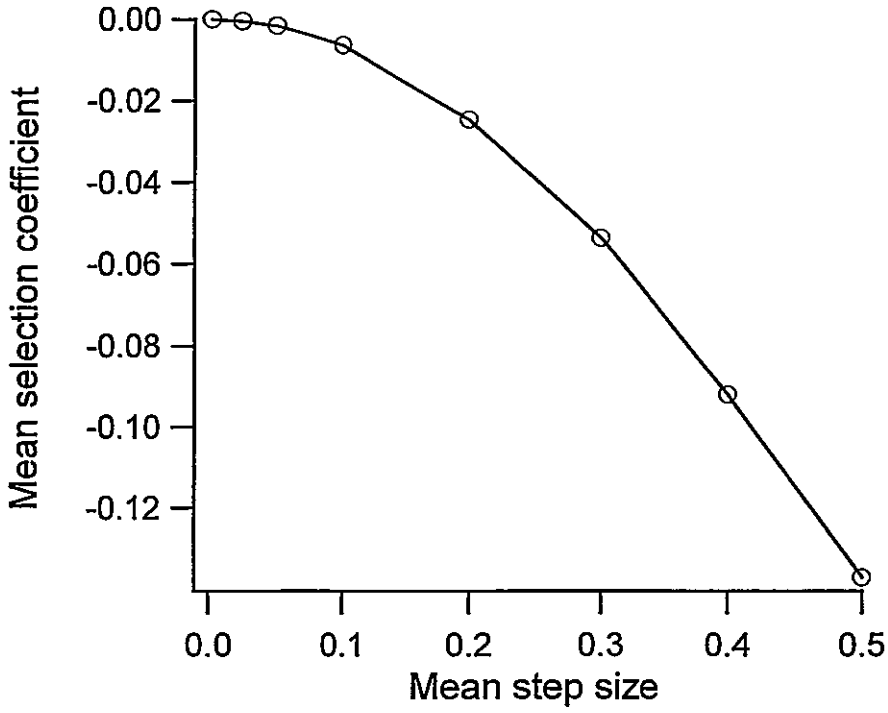
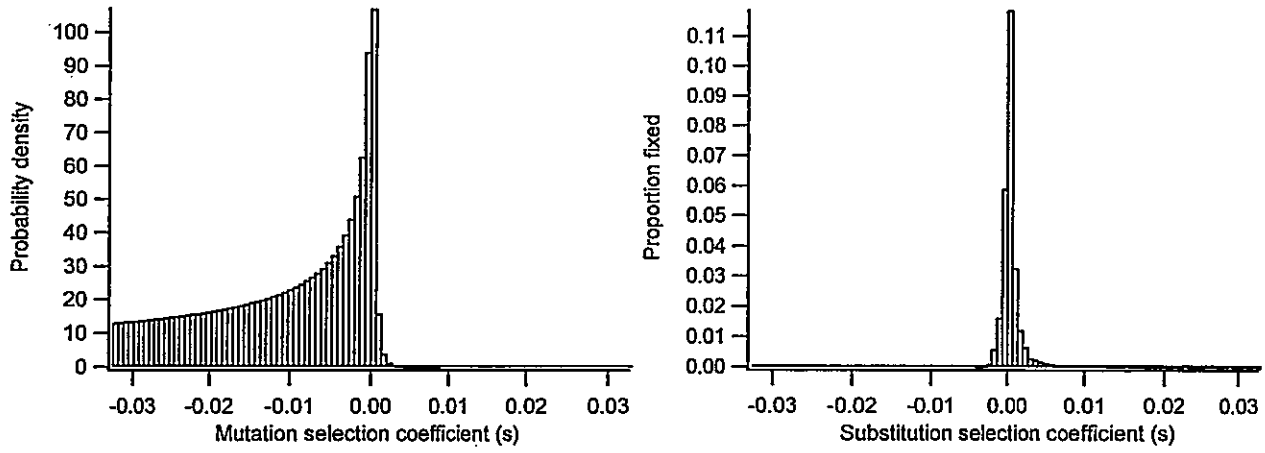
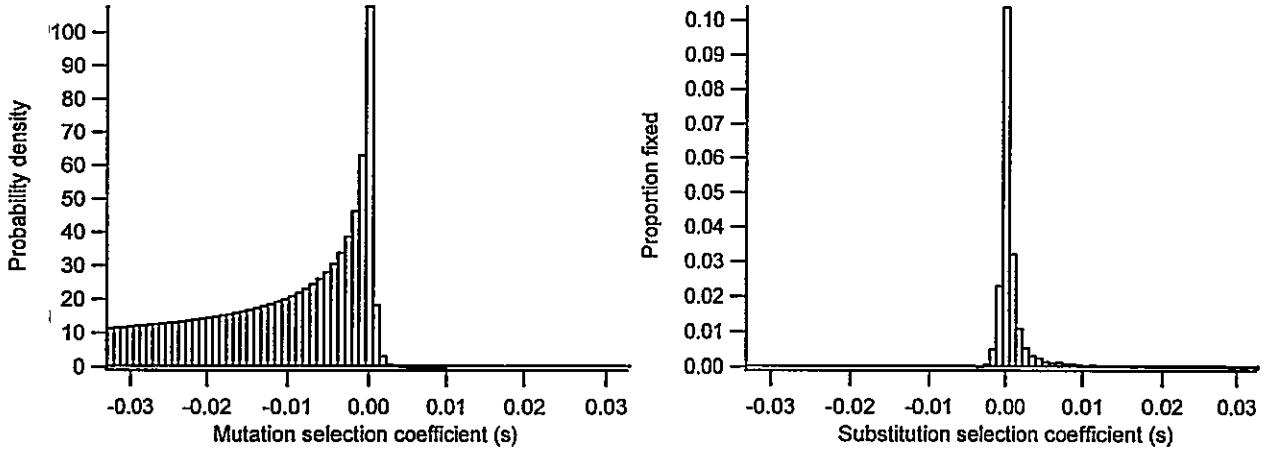


Figure 2. The relationship between the average size of mutations and the mean of selection coefficients. This relation is robust for different dimensions, population sizes, environmental amplitudes, and environmental variability.

a)


Probability density
100
90
80
70
60
50
40
30
20
10
0
-0.03 -0.02 -0.01 0.00 0.01 0.02 0.03
Mutation selection coefficient (s)
Proportion fixed
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.03 -0.02 -0.01 0.00 0.01 0.02 0.03
Substitution selection coefficient (s)

b)


Probability density
100
90
80
70
60
50
40
30
20
10
0
-0.03 -0.02 -0.01 0.00 0.01 0.02 0.03
Mutation selection coefficient (s)
Proportion fixed
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.03 -0.02 -0.01 0.00 0.01 0.02 0.03
Substitution selection coefficient (s)

c)

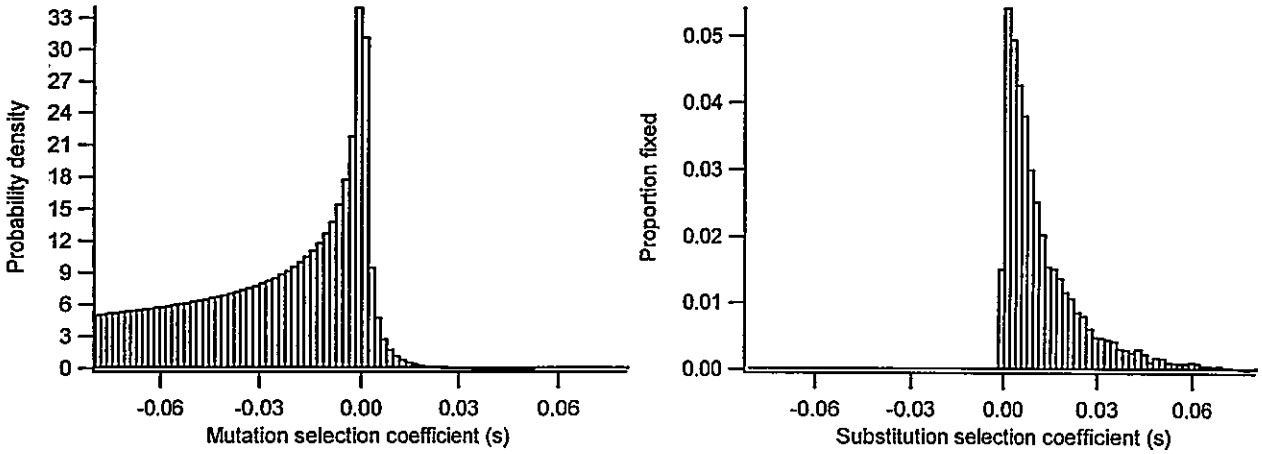

Probability density
33
30
27
24
21
18
15
12
9
6
3
0
-0.06 -0.03 0.00 0.03 0.06
Mutation selection coefficient (s)
Proportion fixed
0.05
0.04
0.03
0.02
0.01
0.00
-0.06 -0.03 0.00 0.03 0.06
Substitution selection coefficient (s)

Figure 3. Probability density of selection coefficients of mutations (left) and the proportion of selection coefficients of substitutions (right) for different environmental variability. a) $\tau = 10^6$, b) $\tau = 5 \cdot 10^5$ and c) $\tau = 10^4$. Parameters used were: number of dimensions $n = 10$, average mutation size effect $r/2 = 0.3$, population size $N = 1000$, amplitude of environmental variability $\sigma_a = 0.35$.

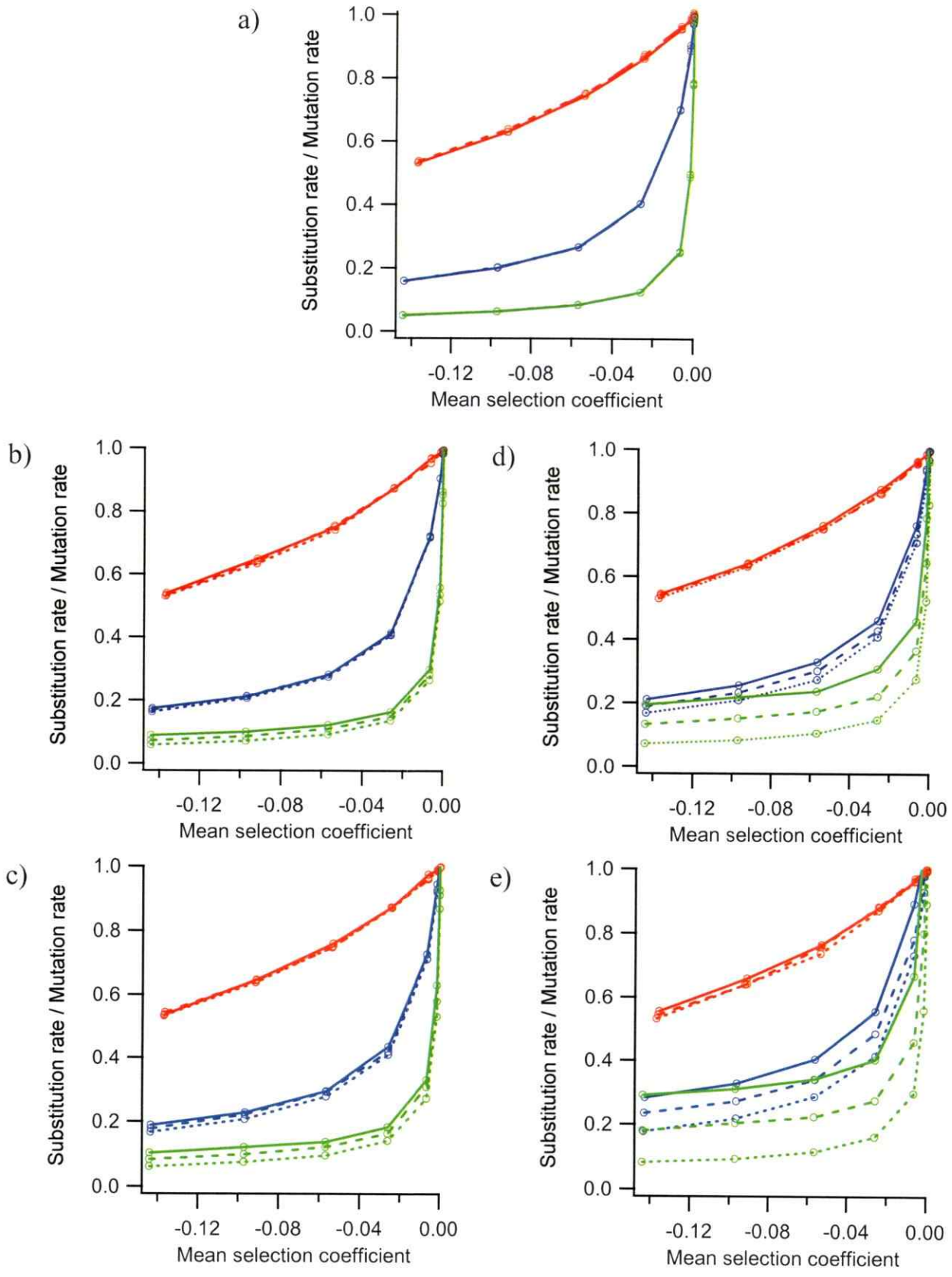
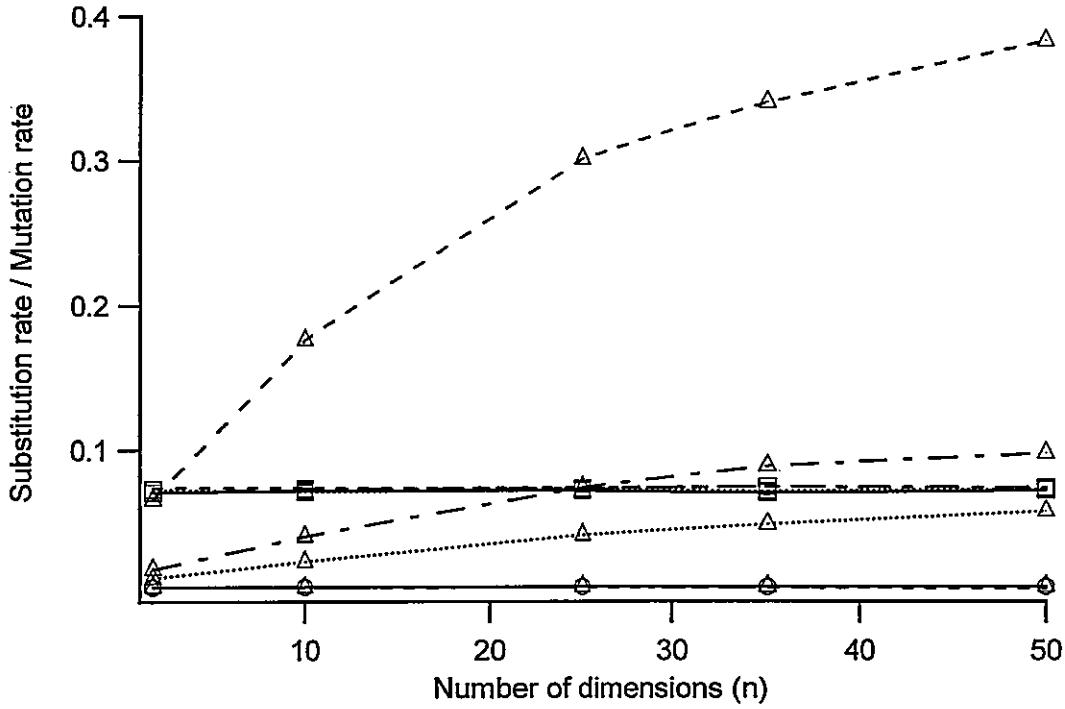

a)
b)
c)
d)
e)
Substitution rate / Mutation rate
Mean selection coefficient
1.0
0.8
0.6
0.4
0.2
0.0
-0.12
-0.08
-0.04
0.00

Figure 4. The ratio substitution rate / mutation rate (k/u) according to the mean selection coefficient of mutations ($r/2$). Parameters used were population sizes $N=10$ (red lines), $N=100$ (blue lines), $N=1000$ (green lines), dimensions $n=2$ (dotted lines), $n=10$ (dashed lines), $n=20$ (solid lines). Points correspond to mutation sizes of Figure 2. Plots correspond to different amplitude and variability of environmental shifts. a) $\sigma_a=0$, b) $\sigma_a=0.425$, $\tau=500\cdot N$; c) $\sigma_a=0.85, \tau=500\cdot N$; d) $\sigma_a=0.425$, $\tau=100\cdot N$; e) $\sigma_a=0.85, \tau=100\cdot N$. Each point corresponds to a simulation yielding 25,000 substitutions.

a)


0.4
0.3
0.2
0.1
Substitution rate / Mutation rate
10
20
30
40
50
Number of dimensions (n)

b)

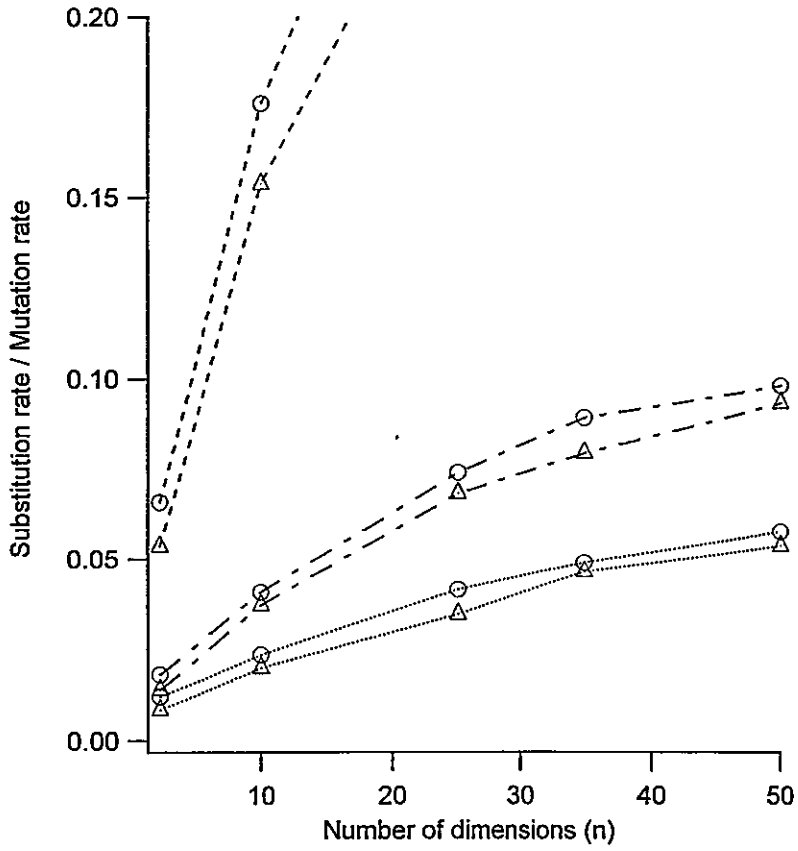

0.20
0.15
0.10
0.05
0.00
Substitution rate / Mutation rate
10
20
30
40
50
Number of dimensions (n)

Figure 5. a) Ratio between substitution rate and mutation rate for effectively advantageous (triangles), effectively neutral (squares) and effectively deleterious (circles) substitutions for different number of dimensions (n). Different traces correspond to different environmental variability: from lower to higher variability lines are solid (stable environment), dotted ($\tau = 10^5$), dashed ($\tau = 5 \cdot 10^5$) and dot-dashed ($\tau = 10^6$). Parameters used were: average size of mutations $r/2 = 0.3$; population size $N = 1000$; number of dimensions: $n = 2$, 10, 25, 35, 50; amplitude of environmental variability $\sigma_a = 0.85$. Every point corresponds to a simulation yielding 20,000 substitutions. b) Ratio between strictly advantageous substitution rate and mutation rate plotted in a) (circles) compared with the same simulations for a higher average of mutation size ($r/2 = 0.5$) (triangles).

CONCLUSIONES FINALES

CONCLUSIONES

En esta tesis se logró implementar un modelo teórico robusto capacitado para simular procesos evolutivos en el marco de la genética de poblaciones y la evolución molecular, bajo un amplio espectro de parámetros. Por primera vez se estudia mediante simulaciones el efecto de la variabilidad ambiental en el FGM, lo cual llevó a la descripción de dos tipos de modelo, que llamamos "Modelo Geométrico Balanceado" y "Modelo Geométrico Variable" (Capítulo 1). Este último modelo permitió también por primera vez estudiar el efecto conjunto del estado estable balanceado y los procesos adaptativos, dos aspectos que previamente han sido estudiados por separado en el marco de trabajo del FGM. Los resultados obtenidos en estos modelos concuerdan en aspectos importantes con modelos desarrollados previamente, pero a su vez difieren en aspectos que permiten explorar vías alternativas de explicación de fenómenos empíricos que han sido difíciles de comprender.

En segundo lugar, el estudio realizado en torno al problema de la creatividad de la selección natural involucró una amplia revisión bibliográfica (Capítulo 2, Sección 1 y Apéndice B) para rescatar un problema que ha durado más de un siglo en la historia de la biología evolutiva y que escasamente ha sido tratado en términos empíricos y de modelamiento formal. Las implicancias del trabajo de modelación (Capítulo 2, Sección 2) conllevan un esfuerzo por afrontar una problemática esencial en la biología evolutiva y creemos haber hecho un aporte en esa dirección.

Justamente en la línea de lo anterior, en el Capítulo 3 fue posible desarrollar un modelo donde la evidencia empírica reciente, que pareció contradecir todas las expectativas

previas, pudo conciliarse con una explicación cualitativa simple y relativamente clara que involucra contemplar el costo de la complejidad de los cambios fenotípicos provocados por las mutaciones genéticas. Así, aunque una mayor pleiotropía génica implica una menor tasa neta de sustituciones, puede implicar también una mayor tasa de sustituciones adaptativas. Como se discutió en el Capítulo 3, este resultado apoya la teoría de la biología evolutiva del desarrollo ("evo-devo") que mantiene que las mutaciones que subyacen al cambio y formación de rasgos morfológicos ocurren principalmente sobre regiones regulatorias más que sobre regiones estructurales de las proteínas. Nuestro modelo predice una más baja tasa de mutaciones fijadas por selección natural sobre sectores del ADN con menor efectos pleiotrópicos, lo que podría explicar la evidencia que se ha utilizado contra la teoría de la evo-devo a favor de la importancia de las mutaciones en genes estructurales (Hoekstra & Coyne 2007, véase Capítulo 3). La resolución de esta polémica es también crucial para la interpretación de la evidencia que se suele entender como prueba de la importancia de la selección natural en la formación de rasgos (Capítulo 2).

Los resultados obtenidos en los diferentes capítulos de esta tesis pueden complementarse para sacar algunas conclusiones generales y perspectivas para desarrollos futuros. En la revisión de las pruebas de adaptación en la naturaleza (Capitulo 2, Sección 1) se describen los posibles fenómenos que los tests genéticos podrían probar. En esta sección se discutió que recientes planteamientos proponen las pruebas genéticas de adaptación podrían no estar realmente probando adaptación sino más bien una sucesión de mutaciones deletéreas y compensatorias que mantienen el status quo. Los resultados del Capítulo 1 tienden a descartar esta hipótesis, puesto que las mutaciones compensatorias en su gran mayoría tendrían coeficientes de selección menores a 1/N, lo que implica que las

mutaciones compensatorias serían indetectables como sustituciones fijadas por selección natural en las pruebas genéticas. Así, nuestros resultados tienden a apoyar la robustez de los métodos de estimación de sustituciones favorables.

Por otra parte, en el Capítulo 2, Sección 2 se mostró que la "adaptación serendípica" de un rasgo, es decir, la adaptación de un rasgo en un régimen dominado por deriva, es posible para rasgos simples (i.e., con un bajo número de características fenotípicas independientes). Si esto lo conectamos con la reciente evidencia de que muchos rasgos cuantitativos difieren entre poblaciones y especies debido sólo a uno o muy pocos loci de rasgos cuantitativos (probablemente un bajo número de genes) de gran efecto (Barton & Keightley 2002, Orr 2005), entonces, podríamos especular que la adaptación serendípica podría observarse en rasgos naturales. A su vez esto implicaría que los tests genéticos de adaptación sobre genes únicos (Capítulo 2, Sección 1) podrían eventualmente adaptarse para probar estadísticamente la creatividad de la selección natural sobre rasgos simples.

Por último, como mencionamos en la Introducción general de esta investigación, las diferentes aplicaciones del FGM que hemos utilizado a lo largo de esta investigación, involucran distintos niveles de organización: organismos, rasgos y genes. Lejos de ser incompatibles, estas aproximaciones pueden ser complementarias. Por ejemplo, una especie S_1, con el mismo número de genes que otra especie S_2, podría evolucionar a menor tasa que S_2, si el grado de pleiotropía promedio de sus genes es mayor (debido al costo de la complejidad, Capítulo 3). Por otro lado, una especie S_1 podría tener un menor número de genes que otra especie S_2, y aún así S_1 ser más compleja que S_2, si el promedio de la pleiotropía de los genes de S_1 es mayor que el de S_2. Esto podría explicar en parte por qué el número de genes aparentemente no se correlaciona con el número de rasgos

morfológicos o número de tipos celulares en un organismo (Szathmáry et al. 2001), y podría ocurrir que organismos con un más bajo número de genes tuvieran un mayor costo de la complejidad, con una mayor carga por retardo (*lag load*).

Cada uno de los modelos desarrollados en esta tesis están sujetos a modificaciones, y pueden ser objeto de revisión en el futuro. De ser así, cumplirían cabalmente el objetivo con el que fueron desarrollados, el cual depende del aporte acumulativo de modificaciones y refutaciones de investigadores con interés en la biología teórica. En particular, es de esperar que la relación entre los distintos niveles de análisis estudiados en esta investigación sean integrados complementariamente en modelos de carácter más general, en cuyo caso el FGM se volvería una herramienta teórica única que permitiría afrontar problemas relativos a la evolución de los seres vivos desde distintos niveles de organización.

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APÉNDICE A

Generation of random vectors in a n-dimensional sphere

The distribution of mutational effect sizes can be critical for the results achieved from the Fisher's (1930) geometrical model (FGM) (Poon & Otto 2000). Empirical data of mutational effects distribution (deleterious, advantageous and neutral) is difficult to obtain, but if we suppose that the distribution of mutation size effects is independent of the degree of population adaptation, the distribution of deleterious mutation of a well adapted population (for example, a population of *Drosophila* flies with high population size) is a good approach to the general distribution (Poon & Otto 2000). The general consensus is that the distribution of deleterious mutant effects on fitness is L-shaped (Keightley 1994, Lyman et al. 1996). Thus, to simulate mutational distributions into the FGM we must be capable to generating random vectors in a n-dimensional space with specifiable distributions (Fig. 1).

Poon and Otto (2000) classified the generation of random vectors in the n-dimensional space with two strategies. The "bottom-up" approach specifies the distribution of mutational length effects along each axis (z_i, with $i = 1, 2, ..., n$) and the total mutation length or vector magnitude (r_j) is leaving unspecified, i.e. it is derived from these components. The "top-down" approach specifies the distribution of total mutational length and leaves unspecified the component distributions along each axis. Both of these approaches are used into the FGM literature. For example, the bottom-up approach is the one used by Poon and Otto (2000), Gu (2007a, 2007b) and Martin and Lenormand (2008), and the top-down approach is the one used by Kimura (1983), Hartl and Taubes (1996,

1998), Orr (1998, 1999) and Welch and Waxman (2003). In both cases, it is very difficult to derive analytically the distribution of the unspecified variables, thus, both methods are not equivalent in the practice and both require computer simulations to make accessible the overall information.

The bottom-up procedure at the simulation level is trivial: sampled numbers $z_1, z_2, ..., z_n$ are generated from a desired distribution and magnitude of vectors is obtained by $r = \sqrt{z_1^2 + z_2^2, ..., z_n^2}$, whose distribution is obtained by numerical simulation. Nevertheless, the top-down procedure is not so simple. According to our knowledge the only one explicit top-down simulation procedure to generate n-dimensional random vectors into the FGM literature is the approach presented by Orr (1998, Appendix 1). This procedure consists of generating random values of hyper-spherical coordinates, i.e. generate random angles $(\theta_1, \theta_2, ..., \theta_{n-1})$ and magnitudes (r) from specified distribution (usually uniform), and afterwards converting these values through the hyper-spherical coordinates transformation (Sibuya 1962) to obtain the rectangular coordinates $z_1, z_2, ..., z_n$. Nevertheless, if we generate vectors sampling angles and magnitudes from uniform distributions, the resultant vectors does not result uniformly distributed in spaces when $n > 2$ dimensions. Instead, the distribution remains with a strong bias toward vectors charged on the poles. For this reason, Orr (1998) stated a correction for the distribution of angles where values of $\cos(\theta_i)$ are sampled from a uniform distribution and angles are obtained by the arccosine of these values. This correction, however, is incorrect for $n > 3$ where vector bias toward the poles remains present and it is extremely biased for higher dimensions. Because simulations into the FGM normally involve $n > 3$ dimensions is

necessary an explicit top-down procedure to generate random vectors in n dimensions. Here we fill this gap.

We modified the method of Muller (1959) to generate uniform random vectors in an n-dimensional surfaces in order to generate uniform random vectors on the complete n-dimensional space and with a specific probabilistic distribution. The method consists in generating n random numbers $Z_1, Z_2, ..., Z_n$ independently according a Gaussian distribution, $Z_i \sim e^{\frac{Z^2}{2\sigma^2}}$, where $\sigma << 1$ can take an arbitrary value, and generate a number r_j according to a desired distribution $r_j \sim m(r)$. Then, rectangular coordinates $z_1, z_2, ..., z_n$ are obtained by:

$$z_i = r_j \cdot \frac{Z_i}{\left(\sum_{i=1}^{n} Z_i^2\right)^{1/2}}$$

Each vector generated by this method will have a uniform distribution into the multidimensional space and a magnitude r_j that follows the desired distribution $m(r)$. Fig. 1 shows results obtained from a space with $n = 5$ dimensions, where there are $\binom{5}{2} = \frac{5!}{3! \cdot 2!} = 10$ pairs of dimensions. In order to test the utility of our method to make simulations in the FGM we reproduce previous results of Fisher (1930), Kimura (1983) and Orr (1998) in the FGM (Fig. 3). This method inherits the speed and generality for any number of dimensions from the method described by Muller (1959, Marsaglia 1972). Furthermore, it avoids making hard spherical coordinates transformations. Therefore, we think that the method is recommendable to be used for the FGM or other computer simulations involving random vectors in a multidimensional representation, particularly

models oriented to obtain results controlling the distribution of vector's magnitudes, e.g. multidimensional random walks and so on.

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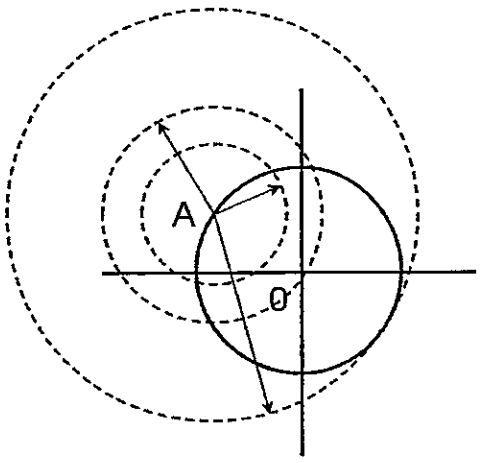

Figure 1: Scheme representing generation of random vectors from a point A displaced to the origin of coordinates O in a two-dimensional space as in the FGM (Fisher 1930).

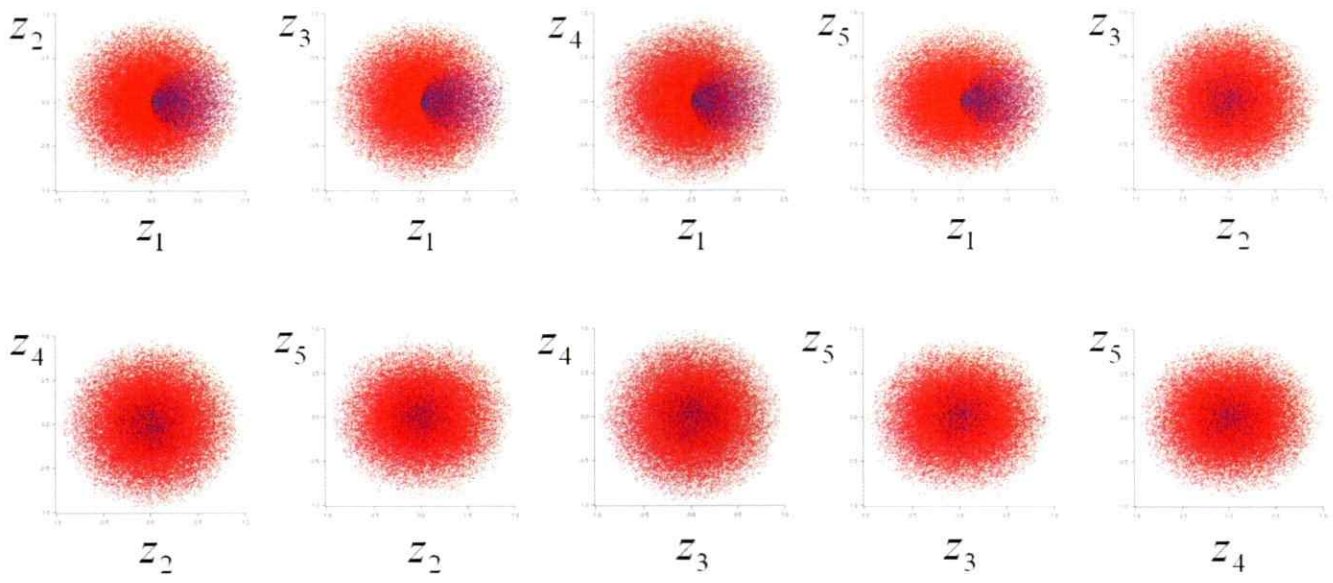

Figure 2: Uniform random vectors generated in a multidimensional space of $n=5$ dimensions. Points represent the extreme point of vectors for the 10 possible combinations of pairs of axes, differentiating between vectors that fall nearer (blue points) and far (red points) to the origin of coordinates into the FGM model (Figure 1). Vectors are uniformly generated from the point $(0.5, 0, 0, 0, 0)$ with a uniform distribution of vector magnitudes $m(r)$ in the range (0,1).

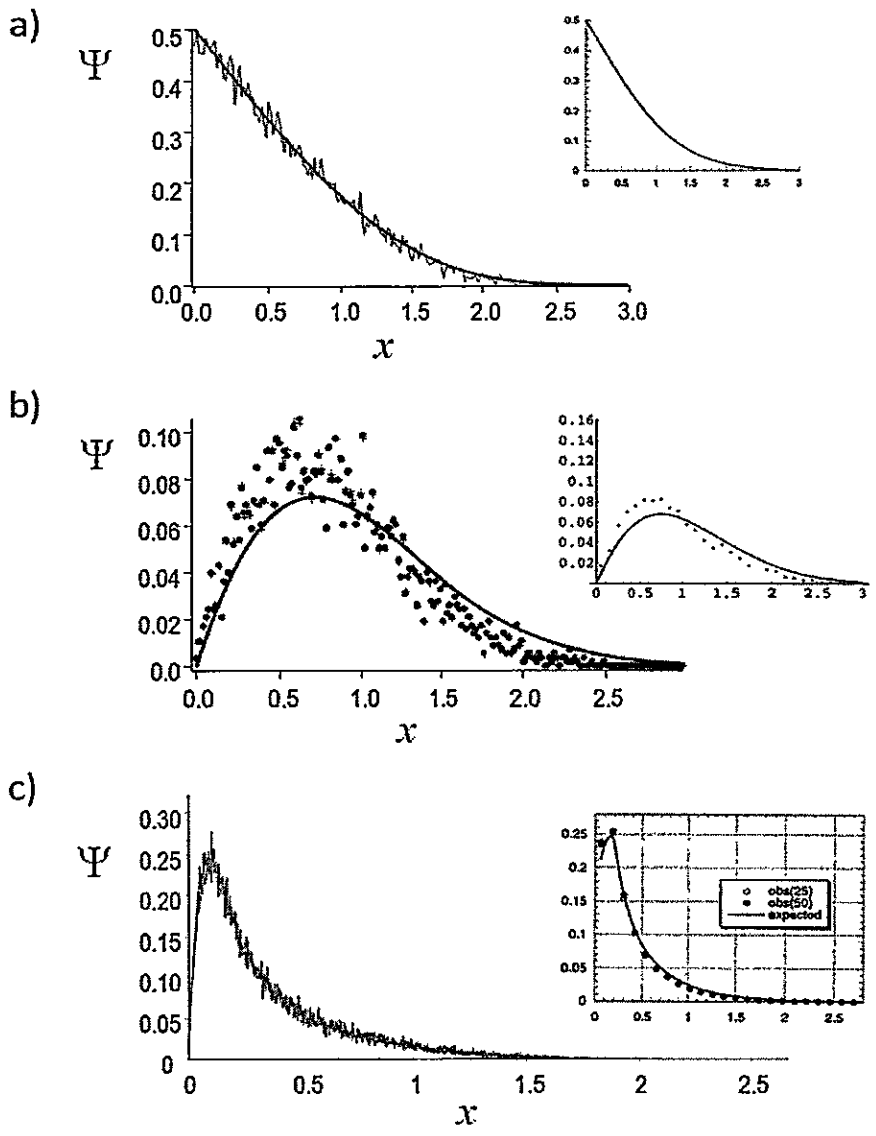

Figure 3: Previous predictions for the FGM obtained using uniform multidimensional random vectors. a) Probability distribution of mutation with size x being advantageous according to Fisher's (1930) prediction. Theoretical prediction (darker line) correspond to $\psi(x) = \frac{1}{\sqrt{2\pi}} \int_x^\infty e^{-t^2/2} dt$. b) Probability distribution of fixation of mutations with size x according to Kimura's (1983) prediction. Theoretical prediction correspond to $\psi(x) = \frac{4x}{\sqrt{2\pi}} \int_x^\infty e^{-t^2/2} dt$. c) Distribution of mutations with size x fixed during an adaptive

bout according to Orr's (1998) prediction. Theoretical prediction corresponds to $\psi(x) = -\frac{1}{\sqrt{2\pi}} \frac{1}{\ln(0.1)} \frac{1}{x} \int_x^{x/0.1} 4\tau \cdot \left(\int_x^{\infty} e^{-t^2/2} dt \right) d\tau$. Independent axes are in standardized units $x = r\sqrt{n} / d$, where d is the distance between the origin of coordinates and the origin of random vectors (i.e, is the distance between the population state and the optimum state in the FGM). Appended plots are taken from Orr (1998) to compare visually with our results.

APÉNDICE B

Probabilistic causation and the explanatory role of natural selection

ABSTRACT: In this study we attempt to face up two questions: i) What can the theory of natural selection explain? and ii) Is there a causal or explanatory model that integrate the natural selection explananda? For the first question, we argue that it has been adjudicated five putative explananda for the theory of natural selection and that four of them could be effectively considered explananda of natural selection. For the second question, we claim that probabilistic causality and statistical relevance to the concept of explanation are good models for the explanatory role of natural selection. We review the disputes about the explanatory role of natural selection and formalized some explananda in probabilistic terms using classical results from population genetics. Most of these explananda have been discussed in philosophical terms but some of them have been homologated and confused. We analyze and set the limits of theses problems.

KEY WORDS: probabilistic causation, explanatory relevance, natural selection explananda, creativity of natural selection

1. Introduction

Since the very beginning of the development of the theory of natural selection theory, there was disagreement about what natural selection (NS) can explain and what not. Nowadays there is a consensus that natural selection can explain the propagation and maintenance of traits in populations. Although these explananda are taken as only one, we will show that they are very different. Henceforth NS explains the trans-generational propagation of traits, the "*Propagation explanandum*" and the trans-generational maintenance of traits, the "*Maintenance explanandum*". In spite of this consensus, there is noagreement about natural selection being able to explain something else. At least three other explananda have been proposed for NS.

The first and the oldest is the question about the origin of organismal traits. NS is usually thought as differential reproductive success of individuals associated to these different individual traits, while the origin of these traits is usually attributed to genetic mutations. From this point of view, it is concluded that NS does not explain the origin of traits and, instead they are pre-assumed by it. We will call this position the "Non-creative View". This viewpoint represents the opinion of many biologists and philosophers from the 19th century to now (see Table 1). Those whom have endorsed this position, argue that NS is a "destructive" force because it simply eliminates or fixes already existent traits, but it does not "create" new traits; this view proposes that the only creative force of evolution is mutation. According to T. H. Morgan (1932) NS merely preserves certain traits but, in the absence of NS, in addition to the known forms of life, a vast assemblage of other types would exist which have been destroyed by selection (Huxley 1936, 1943). The opposed vision is as old as the Non-creative View (see Table 1). This vision asserts that NS is a

creative force because it can generate new traits by the cumulative selection that make probable a combination of mutations which are necessary for trait development and that improbably would be combined together without NS. We will call this position the "Creative View". According to Huxley (1936), Fisher called this process "a mechanism for generating an exceedingly high degree of improbability", and it was for the first time clearly pictured by Muller (1929) as the explanation of the origin of traits. We will call this explanandum the "*Origin explanandum*". Although many scientists who were part in the development of the modern synthesis hold the Creative View (see Table 1), current evolution textbooks allocate none or just a few pages to describe this process (Table 3), and moreover their description is generally metaphoric or expressed in analogical terms. Besides, in spite of this explanandum referring to a probabilistic argument, up to the present there is no a probabilistic formalization of the creative effect of NS.

The fourth explanandum proposed for NS explanans is very recent and we will call it the "*Individuality explanandum*". The key question here is about NS being able to explain why an identified existent individual (namely you, I, Peter or Robert), has the traits it does. Those who defend the affirmative position (the "Positive View", see Table 2), claim that the effect of NS on lineages affects the identity of individual organisms. If the identity of each individual organism necessarily has the property of having been produced by the organisms which in fact produced it ("origin essentialism" thesis), then natural selection explain its identity, and then, its traits (Matthen 1999, 2002, 2003). The other side of the debate (the "Negative View", see Table 2), shares the argument that natural selection cannot explain why particular individuals have the traits they do because NS can only alter population properties but not individual properties. That is, NS cannot explain the

properties of particular individuals. A by-product of this debate has been the proposal that NS can explain why such or such other individual organism exists (although not why it they have the traits they do). We will call it the "*Existential explanandum*". To the best of our knowledge, up to now there is no opposition to this point, but certain arguments could be put against it.

The relationships among Maintenance, Propagation, Origin, Individuality and Existence explananda are by themselves a matter of dispute, and the position about these relationships influence the position about each other. For example, Neander (1995:64) holds that a negative answer to the Origin explanandum (she call it the "Creation Question") entails a negative answer to the Individuality explanandum, while others (Neander 1988:426, Matthen 1999:149, Nanay 2005:7-8) hold that a positive answer to the Propagation and Maintenance question entails a positive answer to the Individuality explanandum. Nevertheless, these arguments do not persuade the partisans of the Negative View (Sober 1995, Walsh 1998, Lewens 2001, Pust 2004). In fact, Walsh (1998) denies that changes in frequency or creation of traits types influence individual traits.

A systematic approach to these issues could be useful to resolve these disputes, or at least to put it in a same ground. Stephens (2007) claims that a resolution of this issue depends on what concept of explanation we assume. Nevertheless, neither it is clear what concept of explanation lead to what conclusion nor what is the most useful concept to analyze this problem. On the other hand, Huneman (2007) has emphasized the difficulty of natural selection explanations to fit a nomological model of explanation. In this study we show that the probabilistic approach to causality and explanation is a good common framework to confront these problems from a comprehensive point of view.

2. Probabilistic causation and statistical relevance

Probabilistic approaches to causation and explanation have a relatively large history. The core aspect of the theory maintains that causes raise the probability of their effects. Thus, the simplest attempt of the probabilistic theory of causation maintains that the factor A is a cause of B if and only if $P(B|A) > P(B|\neg A)$, where $P(B|A)$ represent the conditional probability of B, given A. This Probability-Rising theory has suffered strong criticism (see Hitchcock 2002 for a review). In response to the critics, particularly to avoid the problem of spurious correlation, Reichenbach (1956, sec. 23) and Suppes (1970, ch. 2) proposed a "no screening off" condition, and the more recent Test Situations theory (TS) was proposed by Cartwright (1979) and Skyrms (1980), and generalized by Eells (1991, Ch. 2-4) and Hitchcock (1993). According to the TS theory of probabilistic causation, A causes B if $P(B|A\&T) > P(B|\neg A\&T)$ for every test situation T, where a test situation is a conjunction of factors where is specified those factors that are hold fixed.

On the other hand, coming from the same tradition of probabilistic causation, Salmon (1971, 1984), Jeffrey (1969) and Greeno (1970) developed the Statistical Relevance (SR) model of scientific explanation. The essence of the SR model is that an attribute A is explanatorily relevant to an attribute B if $P(B|A) \neq P(B|\neg A)$. The SR model is similar to the TS theory of probabilistic causation, but implies the specification of an exhaustive and exclusive partition of the possible conditions related to the attribute A and the information about their specific probabilities. We will take the TS theory and SR model as a general causal and explanatory framework to the NS explananda. For our goals

the statistic situations of the TS theory and the partition of the SR model are considered controlled, *ceteris paribus*, and without factors screening off the probabilistic relations considered. This is the usual assumption in the population genetics and molecular evolutionary theories that will be our basis to the formalization (Crow & Kimura 1970). Thus, we can take the essential core of TS and SR by:

$$A \text{ causes } B \text{ if } P(B|A\&T) > P(B|\neg A\&T)$$

$$A \text{ is explanatorily relevant to } B \text{ if } P(B|A\&T) \neq P(B|\neg A\&T)$$

Thus, the explanatory relevance is guaranteed when probabilistic causation is proved. We will attempt to show that all valid explanans for NS theory are understandable from the probabilistic approach to causation and explanation[1]. This attempt is facilitated because the population genetics theory is mainly developed in probabilistic terms (Crow & Kimura 1970).

3. Propagation and Maintenance explananda

Since Darwin's (1858) and Wallace's (1858) foundational works, the only one consensus about the explanatory role of natural selection is that NS explains the propagation and maintenance of traits in nature. Nevertheless, it has been commonplace to take the propagation of traits and the maintenance of traits, as equivalent or implicated explananda (e.g., Neander 1988, 1995a, Walsh 1998). Nevertheless, they stand for two different

[1] For other analyses of natural selection theory as a probabilistic theory see Hodge 1987, 2001, Millstein 2002.

explananda. The difference between selective propagations and selective maintenance comes from the difference between positive and negative (or purifying) selection. NS can explain the *propagation* of a trait (or trait variant) if individuals with a *new* heritable trait have higher fitness than their conspecifics (i.e., if the reproductive success of these individuals with the mutation is higher than the wild type) and their frequency increase into the population[2]. On the other hand, NS can explain the *maintenance* of traits if individuals with any *new* heritable trait have lower fitness than their conspecifics and their frequency does not increase in the population (Fig. 1). Recently, Valenzuela and Santos (1996) and Valenzuela (2000) proposed the use of "substitution" and "fixation" as denoting different concepts in the population genetics because a genetic substitution (a mutation propagated to all populations or species) could not be maintained as "fixed" throughout time. Thus, a mutation propagated until substituing the wild type (i.e., a "substitution") does not have to be maintained necessarily fixed in the population (i.e., a "fixation").

It is clear that the selective explanation of the propagation and maintenance of a trait depend on the positive and purifying selection, respectively. If positive and purifying selection are distinguishable processes then the propagation and maintenance of traits are distinct explananda of NS. That positive and purifying selection are distinguishable processes could be based in three major arguments. First, with the propagation by positive selection consist of a polymorphic transient phase between two times of different states of

[2] In this case we emphasized that NS "can" and no necessarily "explain" the propagation of the trait because, in small populations or for traits with very slightly effect on fitness, the propagation of a trait with higher fitness than the wild type could be explained mainly by stochastic processes as genetic drift if $|Ns|<1$, where s is the selection coefficient of the trait and N the population size.

the population, while the maintenance by purifying selection does not has a polymorphic phase (or it is extremely short). Second, purifying selection cannot be cumulative while positive selection can. Third, although positive selection favoring individuals with the advantaged new trait could seem equivalent to a process of purifying selection disfavoring the disadvantaged wild type, theses processes are not alike at all. Negative selection acts during a short time period whereas the new trait is transiently present into the population. By contrast positive selection acts by a longer time period until the new trait is substituted. In the beginning of the substitution by positive selection, the process seems similar to a purifying selection process: one or few variants are successfully reproduced and the others are unsuccessfully reproduced (Figure 2). Nevertheless, in the purifying process, disadvantageous mutations would have been arisen recently (Figure 2b) and in the positive selection, the disadvantageous trait corresponds to the ancestral wild-type of the population (Figure 2a). That is, to reach this state by negative selection it would be necessary $N-1$ mutations with low fitness, something biologically unrealistic. The very difference between positive and negative selection comes clearer in the assertion that natural selection is a two-step process (Mayr 1962, 1978, 1994, 1997). Positive selection does not entail that there are no individuals eliminated (or without descendants), but it implies that the individuals eliminated are not individuals with recent mutations but individuals with the ancestral wild-type.

It is important to clarify that we should not confuse the positive/purifying selection differentiation with the problem of the "target" of selection (Mayr 1994, 1997). When a selective agent interact with one but not all of the individuals of a population, we could ask if the selective interaction occurs with the fittest individual or with the least fit individual,

i.e., if the target of selection is the advantageous or the disadvantageous individual. By and large the target of selection seems to be thought those individuals with lower fitness or the eliminated individuals (Mayr 1994, 1997), e.g., in predation processes the selective agent interacts with the eliminated, but also the targets can be those subjects with higher fitness, e.g., the elected subjects for mating in a sexual selection process. Positive and purifying selection are processes that can act either if the target of selection is the advantaged or the disadvantaged individual. For example, in the case of purifying selection, individuals with a new disadvantageous trait could be not target of selection because they are not positive target of mate choice or they could be negative target of predation.

The propagation explanandum could be formalized for the example shown in Figure 3a. Natural selection explains that a recent rare mutation that generates a trait T spreads until substituing the wild type if:

$$P(f_t(T) \approx 1 \mid NS \,\&\, f_{t_0}(T) = p) > P(f(T) \approx 1 \mid \neg NS \,\&\, f_{t_0}(T) = p) \qquad (1)$$

It is a classical population genetic result (Crow & Kimura 1970) that if a trait T has a positive selection coefficient s, the probability of substitution of T is $\frac{1-e^{-2N\cdot p\cdot s}}{1-e^{-2N\cdot s}}$, where N is the population size and p is the initial proportion of the trait in population. Thus,

$$P(f_t(T) \approx 1 \mid NS \,\&\, f_{t_0}(T) = p) = \frac{1-e^{-2N\cdot p\cdot s}}{1-e^{-2N\cdot s}}$$

On the other hand, the probability of substitution of a neutral trait (a trait that is not affected by natural selection) is p:

$$P(f(T) \approx 1 \mid \neg NS \,\&\, f_{t_0}(T) = p) = p$$

Thus, (1) is truth since is easy to demonstrate that[3]:

$$\frac{1-e^{-2N \cdot p \cdot s}}{1-e^{-2N \cdot s}} > p$$

always that $s > 0$, i.e., if natural selection acts positively over the trait.

An equivalent formalization could be made for the maintenance explananda. Natural selection explain that a trait T^* is maintained in the population if

$$P(f_t(T^*) \approx 1 \mid NS \,\&\, f_{t_0}(T^*) \approx 1) > P(f(T^*) \approx 1 \mid \neg NS \,\&\, f_{t_0}(T^*) \approx 1) \quad (2)$$

The left term of the inequality is the frequency of a wild type when the population suffers a mutation pressure of a trait with lower selection coefficient than the wild type (and thus, it is constantly purified by negative selection). An also classic result of population genetics is that the disadvantaged trait remains in a frequency of $f(T) \approx \sqrt{\mu}$ in a mutation-selection equilibrium, where μ is the mutation rate of the trait. Thus,

$$f_t(T^*) = 1 - \sqrt{\mu}$$

which is nearly one (unless μ was huge). Thus,

$$P(f_t(T^*) \approx 1 \mid NS \,\&\, f_{t_0}(T^*) \approx 1) \approx 1$$

On the other hand, in the absence of natural selection, i.e., if new mutations are neutral, almost certainly the wild type trait will be substituted by a mutant:

[3] In fact, the function at the left is monotonically increasing and tends to p as s tends to zero.

$$P(f(T^*) \approx 1 \mid \neg NS \,\&\, f_{t_0}(T^*) \approx 1) \approx 0$$

Thus, (2) is immediately demonstrated.

Therefore, these classical results of population genetics show that natural selection can be understood as a probabilistic cause –and it is statically relevant– to the propagation of new advantageous traits by positive selection and the maintenance of traits by purifying selection against new traits.

From the historical point of view it is important to think that the differentiation between the Propagation and Maintenance explananda could be considered part of the causes of the Darwinian revolution. The idea that selective natural forces against new disadvantaged variants maintain the status quo of species was widely spread before Darwin's and Wallace's works (Gould 2002). Thus, the Maintenance explananda is previous to the development of NS theory. The trigger of the Darwinian revolution was the Darwin's and Wallace's assertion that NS can explain the propagation of changes in species, and thus, the change of species over time (Gould 2002).

4. Origin explanandum

The Non-creative View of NS (Table 1) rejects that NS can explain the origin of traits in nature, i.e. it rejects the Origin explanandum. The arguments are of two types: a priori argument and an empirical argument. First, NS only makes sense as acting on an already existent trait, i.e., it has no sense to influence something that does not exist. Thus, by principle, NS cannot have a causal role over the origin of a trait. A second argument is that

NS could have an influence over the origin of a trait in the sense that the environment could make more probable "adaptive" or "directed mutations", i.e. mutations that generate a trait with higher probability if it would be advantageous in such environment. Thus, according to this argument it is an empirical task to resolve is if NS influences the production of a yet nonexistent trait. From the empirical point of view, the existence of adaptive mutation is currently discarded, and thus, selective pressures or adaptive situations for future mutations do not influence the production of such mutations (Lenski & Mittler 1993). This problem was an important issue in genetics and evolutionary biology (see Lenski & Mittler 1993 and references therein), but it could be hardly understood from a non probabilistic approach to causation. Contrarily, the probabilistic account can express this fact as follow:

If the formation of a trait T from a previous structure T_0 involve only one mutational change m_*, then NS is not cause nor it is explanatorily relevant to the origin of trait T ($T_0 \rightarrow T$) because

$$P(T_0 \rightarrow T \mid NS_T \,\&\, m_*) = P(T_0 \rightarrow T \mid \neg NS_T \,\&\, m_*)$$

where NS_{T*} means that the trait T is favored by NS (i.e. $s > 0$).

Thus, the point of the Non-creative View of the origin explanandum is well established when the trait being studied arises from a single mutation[4]. Nevertheless, the majority of interesting traits for biology are not structures consisting only of a single

[4] That is true because the previous state T_0 is taken as a given. However, if the previous maintenance of T_0 is required for that the mutation m_* generates the trait T, and if the previous maintenance of T_0 is explained by NS, then NS explains in part that the mutation m_* generates the trait T.

variation (i.e., a different phenotype due to a single genotypic change), but its evolutionary acquisition involves at least more than one variation cumulated through many generations. The major point of the Creative View is that NS makes more probable the occurrence of types of sequences of phenotypic steps that seem impossible (in other words, extremely improbable) to occur by the random accumulation of changes. This "creative force" of NS has been associated to the ability of NS to explain or cause the "origin", "formation", "generation" or "building" of traits (Table 1). When the origin of a trait T involves more than one advantageous mutation ($m_1, m_2, ..., m_n$), then the probability of this *type of sequence*, with and without NS, should be compared. It is possible to demonstrate that in this case (see Appendix 1):

$$P(T_0 \rightarrow T \mid NS \,\&\, m_1 \cdot m_2 \cdots m_n) > P(T_0 \rightarrow T \mid \neg NS \,\&\, m_1 \cdot m_2 \cdots m_n)$$

Thus, the causal role of NS for the origination of a *type of trait* can be understood as the probabilistic causation of the type of sequence that forms it. Nevertheless, it is important to emphasize two points. First, the argument asserts that NS *can* explain the origin of a trait when it involves more than one advantageous mutation, but it does not asserts that NS necessarily *explains* the origin of a trait when it involves more than one advantageous mutation. The second affirmation is maintained by Huxley (1943), who implicitly assume that it was not possible that two or more advantageous mutations could be substituted by chance. Nevertheless, this cannot be discarded in general in finite populations, and mainly in populations with small or medium size where the role of random drift is important (Crow & Kimura 1970). Second, the argument indicates that the probability of the *type* of sequences that yield that *type* of trait are probabilized if the probability of this type of sequence is lower in a neutral regime where NS does not act.

This allows avoiding the possible probabilistic tautology of the following argument: *the formation of whenever trait that requires a particular sequence of steps to form it will be probabilized if the sequence is probabilized.* For example, the creationist Behe (1996) has criticized the classic argument for the creativity of NS saying that the argument is teleological. According to Behe, the argument assumes the result (a particular trait) and asks the probability of the result if the steps for such result are selected for. Obviously the probability of something is higher if each step to reach to it is probabilized. In order to avoid this tautology, the core point of the origin explanandum could be contrasted with the assertion made by Morgan (1932). Morgan maintained that in the absence of NS all traits of the known forms of life would exist and in addition would exist many others. However, it seems that this is not the case. A proper thought experiment to resolve this point could be a world where all mutations are effectively neutral, i.e., where all new traits do not confer a significantly higher or lower reproductive success to the possessors[5]. Then: What kind of traits would be arisen? The "creativity of NS" argument for the Origin explanandum is that the probably that any known trait would be arisen without NS is much lower than the probability with NS. Thus, the Origin explanandum could be reformulated by the assertion that the probability that the types of traits observed on the Earth would be arisen without NS is lower than with NS. The Darwinism's faith on the Origin explanandum is supported by the extremely intricate organization and complex order of the structure and functionality of traits whose integration and coordination with environmental cues confer to them the aspect of design (traits usually called "complex adaptations"). Nevertheless, it is important

[5] The experiment also could be a world where all species have a small population size, such that $|Ns|<1$ for all mutations (i.e. the effective neutrality of all mutation is always guaranteed).

to note that at least four other possible scientifically valid and non negligible explanations of these organismal features could be responsible for this phenomenon as wll.

First, neutral evolution could explain the arising of complex traits. For example Stoltzfus (1999) proposed a model where complex and intricate traits that "appear to be adaptations" arise, not by the classical model of beneficial refinements but, instead, by a repetition of neutral steps. Thus she criticized common assumptions like the one of Brandon (1990:175): "It is worth noting that presumably no serious biologists think that other evolutionary mechanisms [i.e., other than natural selection], such as drift or pleiotropy, can produce complex and intricate traits that appear to be adaptations." Recently, Lynch (2007a) defended the unavoidable role of non adaptive forces of genetic drift and mutation for the explanation of a large set of evolutionary phenomena as genomic architecture, gene structure, and developmental pathways (Lynch 2007b). Second, it has been proposed that ordered traits with a coordinated stability and order could arise spontaneously in network regulatory systems like genetic or metabolic systems (Kauffman 1991, 1993). Thus the central regulatory and organizational features of cell types and cellular differentiation could be the spontaneous result of systemic relations in networks that meet certain basic features, or the by-product of the increasing in complexity (e.g., the increasing of gene number; see Kauffman 1991, 1993), which in turn could be merely due to passive trends (Wagner 1996, Carroll 2001). That is, if the increasing of gene number is a passive non-adaptive trend, and this is the cause of the major organization of cell types (Kauffman 1991), then NS is not relevant to the explanation of the major organization of cell types at all. Third, a similar spontaneous tendency to the order is characteristic of thermodynamic systems permanently far from equilibrium (Prigogine & Stengers 1984).

Then, because living beings are systems thermodynamically far from equilibrium they have an inherently ordered developmental dynamics that cannot be neglected (Brooks & Wiley 1986, p. 23). Fourth, intracorporeal selection has been historically claimed as an agent of structural and functional phenotypic organization (Gould 2002). Somatic selection (also called "epigenetic selection" or "developmental selection") has been proposed as a mechanism that could explain functional patterns without central coordination of elements (West-Eberhard 2003). Emergent functional properties finely tuned by local adjustments could arise by somatic selection, where spontaneous order or self-organization is the consequence of epigenetic selection processes (Kauffman 1993, West-Eberhard 2003, ch. 3).

Thus, a good challenge for the Origin explanandum of NS is the suggestion that similar types of traits to the known could arise in a neutral world through some of the four previous possible mechanisms for the emergence of organized systems. Nevertheless, this challenge is faced with a long history of skepticism about the possibility to contrast chance with NS in the trait formation, exemplified in the Mayr's words:

> "When one attempts to determine for a given trait whether it is the result of natural selection or of chance (the incidental byproduct of stochastic processes), one is faced by an epistemological dilemma. Almost any change in the course of evolution might have resulted by chance. Can one ever prove this? Probably never." (Mayr 1983).

Interestingly, a new kind of anti-Creative View seems to be arisen (Reid 2007, Badyaev 2008), which seems does not negate that NS *can in principle* explain the increasing of complexity and origin of new traits by the "linear exaggeration of complex structures" (Badyaev 2008), as in the artificial selection, i.e. they does not criticize the

Origin explanandum in a priori terms. Instead Reid (2007) claims that NS *cannot in fact* explain the complexity, trends and diversification or organisms. On the contrary, NS must be relaxed to enable these processes (Reid 2007, Badyaev 2008). In probabilistic terms, if this was the case NS would be explanatory relevant but not a cause of these processes.

5. Individuality and Existential explananda

Beside of the propagation, maintenance and origin explananda, in the last thirty years of discussions in philosophy of biology two curious and metaphysical explananda have been added, namely, the issue whether NS can explain, for a certain individual, *why that individual has the traits it does*; and the issue whether NS can explain, for a certain individual, *why that individual exists*.

Can NS give an account –to some extent, at least– for the traits of individuals? Previous sections would seem to provide us with a rather straightforward answer: yes, of course that NS can help to explain the traits of individuals, whenever the traits in question are the result from a cumulative selection process –indeed, as long as most steps are involved in the process in which a given trait arise, more is what NS can do for its explanation. This is probably the case for your eyes: in so far as yours eyes result of a long evolutionary process, which for sure involved too many mutation, propagation and maintenance episodes, to this extent is that the NS process is involved in the explanations of your eyes.

However, according to certain philosophers things are a little bit more complex that they firstly can look (Table 2). The issue began some few decades ago, concerning initially

with certain passages of the Sober's (1984) book *The Nature of Selection*, such as the following:

"Natural selection does not explain why I have an opposable thumb (rather than lack one). This fact falls under the purview of the mechanism of inheritance (Cummins 1975). There are only two sorts of individual level facts that natural selection can explain. It may account for why particular organisms survive and why they enjoy a particular degree of reproductive success. But phenotypic and genotypic properties of individuals –properties of morphology, physiology, and behaviour– fall outside of natural selection's propriety domain".

..."The frequency of traits in a population can be explained by natural selection, even though the possession of those traits in the population cannot. This reflects the fact... that selectional explanations, unlike developmental ones, do not explain population level facts by aggregating individual level ones. Selection may explain why all the individuals in the room read at the third grade level, but not by showing why each individual can do so". (Sober 1984: p.152)

Karen Neander (1988, 1995, 1995), followed later by others (e.g., Nanay 2005) argued that Elliott Sober (1984), predated for someone and followed by others (e.g., Cummins 1975, Dretske 1988, Dretske 1990) holds a negative view respecting to the role that NS can play in the explanation of adaptations of individuals. Key in the debate that followed is that under the label "negative view", Neander puts together –and sometimes treated as equal– two very different things. First, *the denial that NS can explain how certain types of traits can become into existence*, e.g., how is that for things such as your eyes, being so tricky and complex, was possible arise throughout a purely natural process. The first thing that Neander puts under label "negative view" and attached to Elliott Sober and company is the denial that Darwin and Wallace really answered the old question of the

origin or "creation" of adaptations, to which the natural theology answer with God, a Divine Designer (Paley 1802, for a classical locus). In brief, this is what we have called the "Non-creative View" of NS, regarding to the "Origin explanandum".

The second thing to which Neander refers with the label "negative view" is the denial that NS can explain, for a certain individual, *why that individual has the traits it does*, i.e., the denial that NS can explain, partially although, why you have five fingers in each of your hands, or, say, why Peter has a heart. Since that much about the debate which originated from and followed to publication the Neander's papers was concerned with this question, we'll call this issue the "*Individuality explanandum*", and the affirmative an negative response to it the Positive and Negative View, respectively (Table 2).

For a while, these two things were being discussed together, thus generating a lot of confusion and misunderstanding. However, since Walsh (1998), it was possible to begin discern, every time with more resolution, between they. Whereas the Negative View (the second denial) was effectively proposed and supported by Cummins, Sober and Dretske, it is not clear at all that the same goes with the first denial –rather, it looks as if Cummins, Sober and Dretske simply never considered the Origin explanandum. Neander, by its side, despite arguing vigorously that NS effectively can help answer both the Origin explanandum and the Individuality explanandum, mainly made the case only for the first (Neander 1995a,b).

Putting things as now they are, we have to consider whether NS can or cannot help to explain, for a certain individual, why that individual has the traits it does. A key step in this issue is settle the question in a contrastative epistemological framework, whatever

means simply that when one ask why regarding something aspect, one really ask *why this* aspect *rather than other*. A classical example will be illustrative enough: "Why did Robert rob the bank?" can be understood in several different ways: i) "Why did *Robert*, rather another person, rob the bank?"; ii) "Why did Robert *rob* the bank, rather than have made another in it?; or iii) "Why did Robert rob the *bank*, rather than other place?

The contrasting context becomes especially important to clarify these explananda. Thus, an explanans must explain both the occurrence of the explanandum and the no occurrence of the contrasting context of the explanandum (a counterfactual situation). It is possible to account for a probabilistic approach to the contrastative nature of explanations. For example, in the case iii), if that "the bank is the only place with considerable money in the town" is the explanans (E) that "Robert robs the bank" (e) rather than "Robert robs other place in the town" (the contrasting context, CC), then must be satisfied that:

$$P(e|E) \neq P(e|\neg E)$$

(that is, the probability that Robert robs the bank given that the bank is the only place with considerable money in the town is different to the probability that Robert robs the bank given that also there are others places, e.g. the store, with considerable money in the town). But also that:

$$P(CC|E) \neq P(CC|\neg E)$$

(that is, the probability that Robert rob the store given that the bank is the only place with considerable money in the town is different to the probability that Robert rob the store given that also the store has considerable money in the town).

Now, if we assumed this contrastative epistemological framework, the question "why a particular individual –*a*– have a certain trait –F–?" can be interpreted in two ways: i) why the particular individual *a*, rather than another individual, has the trait F; and ii) why the particular individual *a* have the trait F, rather than another trait, say, F*. As much there are other individuals beside *a* that have the trait F, the question i) doesn't look of particular interest. On the other hand, if we consider the question ii), with it we enter in certain metaphysical issues relatives to personal identity, and in particular, the *origin essentialism thesis.*

Essentialism, in general, is any view that takes certain facts about an individual to be necessary for the identity of that individual. So, someone essentialist respect to certain traits of a species, says, the colour blue to species X, is someone that thinks that if this organism –actually a member of species X–were not to be blue, then this organism would not be an organism of the species X.

So, the origin essentialism thesis is the thesis that takes certain fact concerning to the origin of an individual to be essential to the identity of that individual. Usually, this thesis is interpreted as meaning that, *inter alea*, the parents of an individual are necessary to his identity, so an individual with another parents that his own, it is nothing but an impossible metaphysical fantasy –an individual with another parents than he actually has, is a different individual than he actually is, he is not the same individual but with different parents, that is not possible.

Now, what this have to do with the problem whether NS can help explain why particular individuals have the traits they do? Indeed, if we assume that the traits in

questions are those transmitted from parents to descendent, then it is clear that, by the origin essentialism thesis, an individual with different traits from the traits he has is an individual with another parents than his own, and so, an impossible situation. Simply it is not possibly, for a certain individual, to have traits distinct from the traits he actually has. This imply that question ii) lacks of a contrastative clause, and a fortiori, by the contrastative epistemological framework, that it is not a question at all. For this reason, NS –and any else– cannot help to explain why particulars individuals have the traits they do. The question is nonsense (and the probabilistic account to the contrastative nature of explanations inapplicable). So, we must conclude that NS cannot answer the Individuality explanandum.

A by product of the later discussion was the question about if NS can explain that particular individuals exist rather than do not exist (the Existential explanandum). Because NS determines in part what of all possible particular individuals that could have been existed and reproduced effectively existed and reproduced (Walsh 1998), then NS can explain the existence of the particular individuals. Although there are not known philosophical oppositions to this idea some problems could be put against. Brooks & O'Grady (1986) argued that nonexistent items "cannot be cited in explanations of the etiology of the species which exist" (p. 84) and that arguments of the type "the frog is green because red, yellow, and blue frogs have long been devoured" require a "negative space" of "other possible species" which are non-evidential and cannot be taken account in scientifically acceptable explanations (pp. 84ff). This argument is subsumed under the wide range of discussions about the counterfactual approaches of causality and explanation, and we will not enter in this discussion. One thing that seems evident is that the Origin and

Existential explananda necessarily allude to counterfactuals and that the contrastative nature of explanation requires an explicit reference to counterfactuals in order to consider an explanation complete.

Another problem with the Existential explanandum is that the contingent and historically specific nature of the genealogical process (meeting, mating, meiosis, fertilization, vital dangers, and so on) make the individuality of particular organisms extremely fragile (Parfit 1986). Thus although NS could influence the curse of contingent facts that probabilize the arising of organisms with certain traits (the Origin explanandum), the influence over the existence particular individuals could be so negligible in light of the strong effect of other contingent factors. So, to consider it as an explanandum of NS could be far-fetched. Indeed a probabilistic estimation to the contrasting context of the existence of particular individuals seems to be implausible.

6. The plurality of natural selection explananda

We have shown that the NS can be understood as a probabilistic cause. Using other concepts of cause, some authors have been questioned that NS can be understood as a cause (Walsh 2000, 2002, Matthen & Ariew 2002, Brunander 2007). Nevertheless, the theory was originally constructed as a causal theory (Hodge 2001) and just like that is generally understood in evolutionary biology until now. The probabilistic formalization of the population genetics and the usual use of concepts as "evolutionary force" are a good reflect of the convenience to the probabilistic causality and statistical explanatory relevance account for the natural selection theory.

It could be useful to make a distinction between the theory of natural selection (TNS) and the natural selection (NS). The TNS is not a theory that only claims that NS exist, but NST claims that NS is a causal factor that, in conjunction with a source of inheritable variation, can explain and in fact explains a certain set of types of explananda. As we argued, there are at least four of these types of explananda, then the delimitation and understanding of the TNS requires the specification and understanding of these explananda and how they are related.

As it could seem evident, the scope of the NS explanatory aspirations depends on the relationship between explananda. The Propagation and Maintenance explananda clearly are pre-requisites to the Origin explanandum. Nevertheless, Propagation explanandum cannot count by itself, because if a trait is propagated and fixed by NS, that does not guarantees that the trait maintains throughout time. For example, an antibiotic resistance could be fixed in a population of bacteria in an environment with antibiotic, but if the environmental pressure ceases (the environment is without antibiotic) the antibiotic resistance probably will be replaced[6]. In turn, the Maintenance explanadum has the Propagation explanandum as a logical requisite. Although this explanandum does not imply that NS can explain why particular individuals have the traits they do (the Individuality explanandum) because there are not a contrasting context with sense, these explananda implies that only a sub-set of the huge number of possible particular individuals could effectively exist, and thus indirectly it seems to affect (although as one among very many

[6] Indeed, the antibiotic resistance in bacteria has a fitness cost (e.g., Schrag et al. 1997, Levin et al. 2000), but in general if selective constraints are relaxed the trait will be substituted by random mutation and drift (Kimura 1983, see also Valenzuela 2000).

others factors) the probability of the existence of particular individuals (the Existence explanandum). Finally, the Origin explanandum depends of a cumulative effect of propagations and maintenance of changes, but they must be directed toward some integrative functions or structures, such that the combination of the changes is coordinated into an organized new trait.

7. A very brief historical note of NS explanda

The idea of selection as a force that preserves the *status quo* (the stasis of species) was common before Darwin and Wallace (Gould 2002, ch.1). Thus, the Maintenance explanantum is a pre-Darwinian concept. Furthermore, the Propagation explanandum of Darwin and Wallace also has some predecessors (as Patrick Matthew (1790-1874) and William Charles Wells (1757-1817), see Gould 2002). Nevertheless, the Origin explanandum seems to be original of Darwin (and apparently not of Wallace, see Ayala 2007), and it really begins to be clearly explained with the work of neo-darwinians biologist, being Muller (1929) the first in explaining it in a clear probabilistic form (although with some antecedents as Weismann 1891 and Roux 1881 but mainly in terms of intracorporeal selection; see Gould 2002). The Individuality explanandum emerged just with the Sober's (1984) work. The Existential explanandum emerged as a response to the Sober's original claim (Walsh 1998), but it was furher developed by Matthen (1999, 2002, 2003) and Pust (2001, 2004). We see the history of the NS explananda as a history of emergence and divergence (differentiation). That is, explananda arise confusedly as a single

explanandum and posterior analyses discovered that the issue deals with different explananda.

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APPENDIX 1:

If the formation of a trait T from a previous structure T_0 involve n mutations ($m_1, m_2, ..., m_n$), then NS is explanatorily relevant to the formation of T* if

$$P(T_0 \rightarrow T \mid NS \,\&\, m_1 \cdot m_2 \cdots m_n) > P(T_0 \rightarrow T \mid \neg NS \,\&\, m_1 \cdot m_2 \cdots m_n) \quad (3)$$

where NS means that mutations m_i are selectively favourable (i.e. $s_i > 0$) and $\neg NS$ means that mutations m_i are selectively neutral (i.e. $s_i \approx 0$)

Let us assume that the time between mutations is higher than the time to substitution or loss of fixations in the population. Then (Crow & Kimura 1970),

$$P(T_0 \rightarrow T \mid \neg NS \,\&\, m_1 \cdot m_2 \cdots m_n) \approx \left(\frac{1}{N}\right)^n$$

In turn (Crow & Kimura 1970):

$$P(T_0 \rightarrow T \mid NS \,\&\, m_1 \cdot m_2 \cdots m_n) \approx \left(\frac{1-e^{-2s}}{1-e^{-2Ns}}\right)^n$$

Then, because $\frac{1-e^{-2s}}{1-e^{-2Ns}} > \frac{1}{N}$, (3) is demonstrated.

It is important to mention that would be a probabilistic tautology if we assume that all mutations fixed in the trait formation had positive selection coefficients. If this is the case is an empirical, not a priori, matter.

Table 1: Papers or books that defend the "Non-creative View" or the "Creative View" about whether natural selection explains why exist (or existed) individuals that have a certain trait (the "Origin explanandum").

Non-creative Views of NS	Creative Views of NS
Mivart (1871)	Darwin (1859)
Cope (1887)	Weismann (1896)
Eimer (1890)	Muller (1929)
Morgan (1905)	Fisher (1932)
Osborn (1909)	Fisher (1934)
Punett (1911)	J. Huxley (1936)
Hogven (1930)	J. Huxley (1943)
Morgan (1932)	Simpson (1944)
Uexküll (1945)	Simpson (1947)
Berg (1969)	Fisher (1954)
Grene & Glicksman (1974)	Dobzhansky (1954)
Cummins (1975)	Lerner (1959)
Nagel (1977)?	Kimura (1961)
Wassermann (1981)	Mayr (1963)
Maturana & Varela (1984)	Ayala (1970)
Endler (1986)	Dobzhansky (1974)
Brooks & O'Grady (1986)	Gould (1977)
Dretske (1988)	Dawkins (1986)
Saunders (1989)	Neander (1988)
Dretske (1990)?	Maynard-Smith (1989)
Gilbert et al. (1996)	Millikan (1990)
Arthur (1997)	Godfrey-Smith (1992)
Mahner & Bunge (1997)	Ayala (1994)
Maturana & Mpodozis	Doolittle (1994)
(1992, 2000)	Neander (1995a,b)
Arthur (2000)	Walsh (1998)
Walsh (2000)	Ayala (2000)
Muller (2003)	Gould (2002)
Muller & Newman (2003)	Nanay (2005)
Ariew (2003)	Frober (2005)
Reid (2007)	Ayala (2007)
Badyaev (2008)	Avise & Ayala (2007)

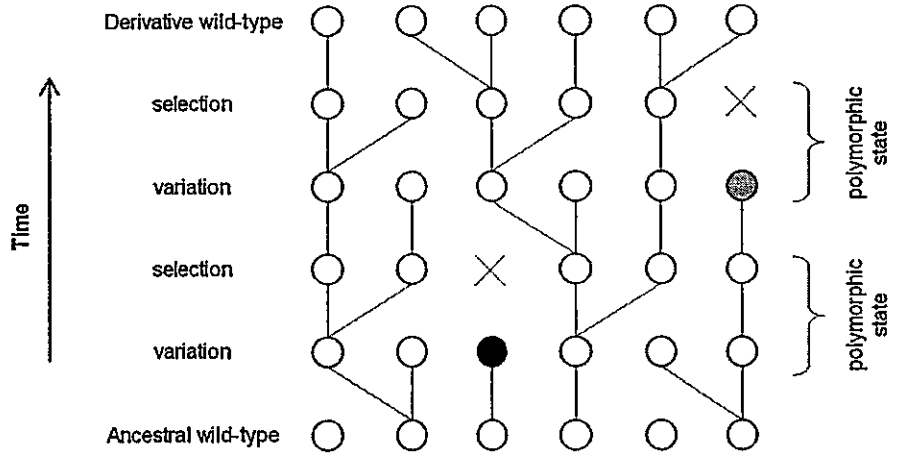
Table 2: Publications that defend the "Negative View" or the "Positive View" about whether natural selection explains why identified individuals have the traits they do (the "Identity explanandum").

Negative Views of NS	Positive Views of NS
Sober (1984)	Neander (1988, 1995a,b)
Sober (1995)	Matthen (1999)
Walsh (1998)	Nanay (2002)?
Pust (2001)	Matthen (2002)
Lewens (2001)	Matthen (2003)
Pust (2004)	Nanay (2005)?
	Frober (2005)?

Table 3: General textbook of evolutionary biology and the number of pages explicitly dedicated to the mention, description or analysis of the creative effect of natural selection.

Textbook	Number of pages
Futuyma (2000)	0
Rydley (1999)	1
Maynard Smith (1989, 1998)	5
Fox *et al.* (2001)	0
Mayr (1963)	2
Freeman & Herron (2001)	0
Strickberger (2000)	2
Soler (ed.) (2002)	0

a)


Derivative wild-type
selection
variation
selection
variation
Ancestral wild-type
Time
polymorphic state
polymorphic state

b)

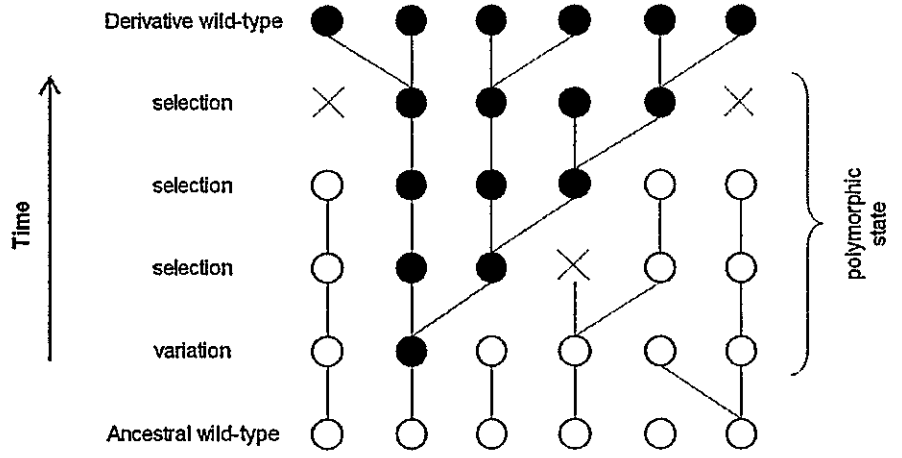

Derivative wild-type
selection
selection
selection
variation
Ancestral wild-type
Time
polymorphic state

Figure 1. a) Maintenance of a trait by permanent processes of negative (or purifying) selection. New variants (full circles) with lower fitness than wild-type (white circles) are eliminated in the short term because its selective disadvantage. Individuals eliminated have recent new mutations. b) Propagation of a trait by a process of positive selection. The new variant (full circles) with higher fitness than wild-type (white circles) are propagated because its selective advantage. Individuals eliminated do not have new mutations.

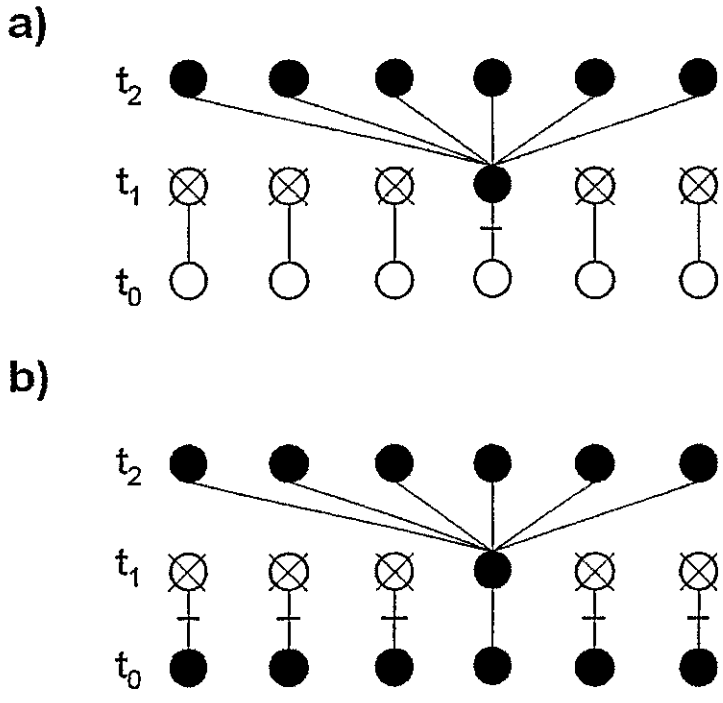


Figure 2. a) A very short process of positive selection: a one generation substitution in t_3 of a new advantageous trait (black circle) arising in t_2 and the elimination of the N-1 ancestral individuals wild-type (white circles) at t_0. b) A thought process of purifying selection: Substitution in t_2 of an ancestral advantageous variant (black circle) and the elimination of N-1 new disadvantageous traits (white circles) arising by mutation in t_2 from the wild-type ancestral individuals of t_0. The steps $t_1 \rightarrow t_2$ are identical both for a) and b), but in b) is supposed a multiple mutation event arising of traits with lower fitness than the wild-type, a situation biologically implausible.

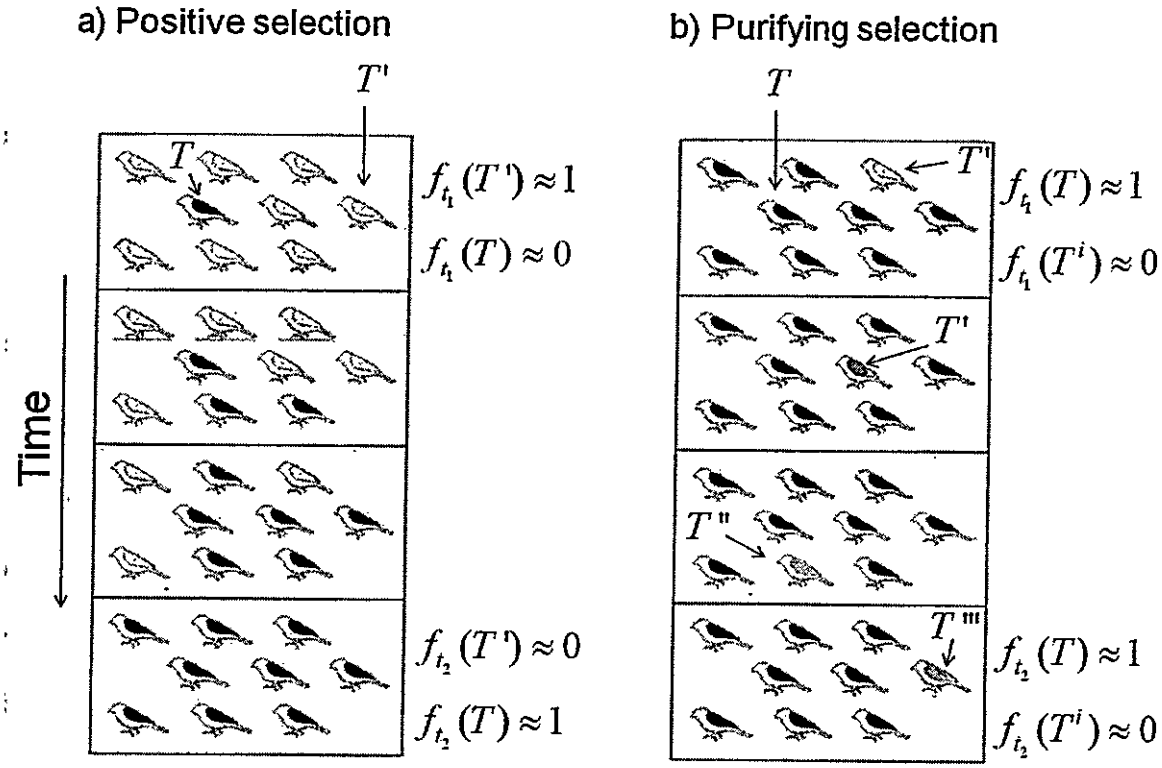


Figure 3: Example of positive (a) and negative (b) selection processes.