Hereditas 131: 227–238 (1999)

Genomic compatibility between two phyllotine rodent species evaluated through their hybrids

LAURA I. WALKER[1], MARIANA ROJAS[2], SERGIO FLORES[1], ÁNGEL SPOTORNO[1] and GERMÁN MANRÍQUEZ[1]

[1] *Laboratorio de Citogenética Evolutiva de Mamíferos*
[2] *Laboratorio de Embriología Comparada, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile.*

Walker, L. I., Rojas, M., Flores, S., Spotorno, Á. and Manríquez, G. 1999. Genomic compatibility between two phyllotine rodent species evaluated through their hybrids.—*Hereditas 131*: 227–238. Lund, Sweden. ISSN 0018-0661. Received October 4, 1999. Accepted December 13, 1999

In order to investigate the genomic compatibility between allopatric rodent species, *Phyllotis darwini* and *Phyllotis magister*, we have studied several cytogenetic and reproductive features of their laboratory hybrids. Of thirty-one pairings between species, only five were successful, producing eleven newborns. Like parents, hybrids had 38 metacentric chromosomes, except for the subtelocentric Y chromosome inherited from *P. magister*. There was almost total C and G band correspondence between homeologous autosomes. However, parental sex chromosomes had different morphology, C and G bands. Ag-NOR bands appeared as small telomeric Ag^+ regions, distributed in four chromosomal pairs of *darwini*, three of *magister* and four homeologous chromosomes of the hybrids. The three forms had similar indexes of NOR activity per cell, in spite of the variability in NOR expression which was always detected. Usually, only one member of parental homologous chromosomes showed $AgNOR^+$; nevertheless, both homeologous chromosomes were active in many hybrid cells. The frequencies of cells that expressed their ribosomal genes in the two homologous or homeologous NOR chromosomes were similar in parental and hybrid cells. These results strongly suggest that ribosomal genes of both parental genomes would function codominantly in the hybrids. The gonad histological and morphometric analyses showed that hybrids conformed to Haldane's rule, since females were fertile and males were infertile. Our results indicate that *P. darwini* and *P. magister* genomes can function in relative harmony and compatibility when they are placed together in their laboratory generated hybrids, suggesting that these species have few genetic differences, probably because they have recently diverged.

L. I. Walker Laboratorio de Citogenética Evolutiva de Mamíferos, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile. P. O. Box 70061-Santiago 7, CHILE. E-mail: lwalker@machi.med.uchile.cl

The normal functioning of any genome depends to a large extent on the epistatic interactions between both sets of autosomal genes and between autosomal and sex-linked genes. In interspecific hybrids, these interactions are disturbed and the compatibility of the two genomes joined in hybrid cells depends mainly on the genetic divergence produced between parental species (COYNE and ORR 1989a; 1997).

We have previously obtained several laboratory hybrids between the Andean rodent species *Phyllotis darwini* and *P. xanthopygus vaccarum*. Although these hybrids reached adulthood, males and females were completely sterile (WALKER et al. 1984). In that case, parental species differed cytogenetically in six chromosomes, amount of constitutive heterochromatin and genome size (WALKER et al. 1984, 1991). Despite the mean number of active nucleolar organizer regions (NORs) were similar in the two species, they were localized in different chromosomal pairs. In the hybrids we found that active NORs were mainly from *darwini* chromosomes (WALKER et al. 1998).

We now report several cytogenetic and gonadal features exhibited by the laboratory generated hybrids between two other phyllotine species: *Phyllotis magister* and *Phyllotis darwini*. *P. magister* inhabits on the mountains of Perú and northern Chile (PEARSON 1958, 1972) and recently, was also collected on the coast of northern Chile (SPOTORNO et al. 1992). *P. darwini* lives only on the coast of central Chile (WALKER et al. 1984). Both species' karyotypes are very similar, differing only in one pair of autosomes and in the sex chromosomes (WALKER et al. 1979).

To evaluate the compatibility between *P. darwini* and *P. magister* genomes, we investigated in adult hybrids the activity of the parental NORs and the histological and morphometric characteristics of male and female gonads. If parental species genomes have relatively few genetic differences, both parental NOR sets will usually express in hybrid cells and adult hybrid gonads will, at least in some cases, reach an organization that will allow gamete production. We found that this was generally the case.

MATERIALS AND METHODS

Specimens

Skulls and skins of all the studied individuals were prepared as voucher specimens and were deposited in the collection of the Laboratorio de Citogenética, Facultad de Medicina, Universidad de Chile (LCM). *P. magister* specimens were collected in the field, at the mouth of the Loa River, II Región, Chile. *P. darwini* animals were members of a small laboratory colony, which descended from specimens collected at Totoralillo, IV Región, Chile.

Pairings

62 intraspecies crosses and 31 interspecies crosses were performed (Table 1). Each pair of each cross was placed in a plastic cage (32 cm × 28 cm × 14 cm) with a bed of shavings. Food pellets and water were provided ad libitum. A natural daylight regime was followed. Cages were checked twice a week and births, litter sizes and deaths were recorded. Since phyllotine females present a postpartum estrus period (WALKER 1988), males were kept in the cage for three to five more days after parturition, and were then removed.

Chromosome analysis

Chromosomes were obtained from bone marrow cells using the conventional in vivo colchicine, hypotonic method, preceded by yeast injection to improve the mitotic index (LEE and ELDER 1980). Metaphase cells were treated with C banding (CROSSEN 1972; SUMNER 1972) and G banding techniques (CHIARELLI et al. 1972). Selected C and G banded metaphases of each hybrid were compared with those from their biological parents and from other specimens of the parental species. The active NORs were detected by silver staining procedures (GOODPASTURE and BLOOM 1975; SÁNCHEZ-RUFAS et al. 1982). To unambiguously identify the chromosomes carrying NORs, we used a sequential AgNOR G-banding procedure (NOR-G banding), developed in our own laboratory. The number and chromosomal distribution of active NORs were then recorded in the hybrids and compared with those found in the parental species karyotypes.

Gonads

Gonads from adult *magister* (1♂ and 1♀), *darwini* (1♂ and 1♀) and hybrids (2♂ and 1♀), were fixed in Dubosq-Brasil fluid, serially sectioned (5μ) and stained either with hematoxilin-eosin or PAS-Schiff. Cross sections were used to perform the morphometric study of the testes. Relative volumes or volumetric densities (VD) of seminiferous epithelium, tubular lumen and interstitial compartment, were calculated. A 10 × 10 ocular grid placed into the microscope eyepiece was superimposed on the slide image. The number of events where the intersections of the grid coincided with any of the above mentioned tissue areas, were recorded and expressed as percentages. Means and standard deviations were calculated and their differences were statiscally evaluated using the Student's t test.

Spermatogenic index (SI) and interstitial cell index (ICI) proposed by GROCOCK and CLARKE (1974), were used to evaluate the functional state of the testes. The SI gives a measure of seminiferous epithelium activity. Values range from five, representing complete spermatogenesis with abundant spermatozoa production, to zero representing the presence of spermatogonia and Sertoli cells only. The ICI (values ranging from five to one) is an assessment of the state of interstitial tissue, based on the relative size of this testis compartment and on the shape of its cell nuclei. The presence of spermatozoa in epididymal and vas deferens lumina were also investigated.

Cellular suspensions of testes were obtained from parental and hybrid adult males, according to the procedure described by EVANS et al. (1964), stained with 4 % Giemsa solution, and the number and diversity of germinal cell types in each, were registered.

For analysis and comparisons of ovaries, we considered the presence of follicles and corpora lutea and the appearance of the ovarian stroma.

Table 1. *Births, aggression and litter size in homologous and heterologous crosses between Phyllotis darwini and Phyllotis magister*

Type of cross male × female	Number of pairs	Pairs with births N (%)	Pairs with aggression* N (%)	Litter size $\bar{X}$ (N)
darwini × *darwini*	58	40 (69.0)	5 (8.6)	4.2 (33)
magister × *magister*	4	2 (50.0)	0 (0.0)	3.5 (2)
darwini × *magister*	14	1 (7.1)	5 (35.7)	2.0 (1)
magister × *darwini*	17	4 (23.5)	2 (11.8)	2.3 (4)

*Pairs where one member was found dead and with visible wounds within 30 days of the pairing date.

RESULTS

Pairings

The number of *darwini* × *darwini* pairings reported here is higher than that registered for *magister* × *magister* crosses (Table 1). *P. darwini* species has been reproduced in our laboratory from many years, so much data about its biology is now available to us. However, *P. magister* specimens were specifically collected for this study and they were preferentially used for interspecific crosses.

Although for a thorough comparison of the reproductive characteristics of these two phyllotine species, it is really necessary to produce a large number of *magister* intraspecific crosses, the data we obtained highlight that under laboratory conditions, *magister* is less sexually active (pairs with births, Table 1) and less aggressive, than *darwini* (pairs with aggression, Table 1). The recorded *P. magister* litter size, also seems to be smaller than that reported for *P. darwini* (Table 1; WALKER et al. 1984).

From a total of 31 crosses between the two species, only five were successful, producing eleven newborns. Of these, six reached adulthood and were seemingly healthy. The hybrid average litter size was 2.2, which differed considerably from that observed in the intraspecies crosses (Table 1). In crosses between *magister* males × *darwini* females, births occurred more frequently and aggressive behaviour, less frequently, than in reciprocal pairings (Table 1). So, this type of pairing produced nine of the eleven obtained hybrids. The other two specimens originated from the single parturition that occurred in the male *darwini* × female *magister* pairings.

Karyotypes

Diploid and fundamental numbers, previously described for *magister* and *darwini* species (2n = 38, NF = 76; PEARSON 1972; WALKER et al. 1979), were confirmed. As expected, all hybrids had 38 metacentric or submetacentric chromosomes, except for the Y chromosome of the ♂ *magister* × ♀ *darwini* males, which was telocentric, the same as of *magister* fathers.

Previous studies have shown that all the *darwini* chromosomes and *magister* autosomes have very small pericentromeric C bands (WALKER et al. 1979, 1984). However, the *magister* sex chromosomes show a distinctive C band pattern, with the submetacentric X chromosome having a large C band in the proximal region of its short arm and the telocentric Y chromosome beeing entirely heterochromatic (Fig. 1a; WALKER et al. 1979). In accordance with these parental C band patterns, the cytogenetically studied hybrids showed small pericentromeric C bands in all autosomes and in the X chromosome inherited from *darwini* mothers. The other hybrid females X chromosome had a large C band in its short arm and the telocentric Y chromosome, inherited from *magister* fathers, was heterochromatic (Fig. 1b).

Because the G banded karyotypes of *magister* and *darwini* are very similar (WALKER et al. 1979), almost all the homeologous hybrid chromosomes showed complete G band correspondence. The exceptions were a medium size autosomal pair (No 10) and the sex chromosomes (Fig. 1c), confirming that these are chromosomes unique to each parental species karyotype (WALKER et al. 1979). Slight differences between the short arms of homeologous chromosomes 7, can also be observed in early metaphases.

The number of AgNOR bands varied in the cells of parental species and their hybrids, although some regularities emerged from our data. They were always observed as small Ag positive regions, located at telomeric sites of three or four different chromosomal pairs in the cells of both parental species, usually on a single member of homologous pairs. Thus, in metaphases of four *darwini* individuals (3♂ and 1♀), four chromosomal pairs carrying active NORs were observed (Nos 3, 7, 9 and 12; Fig. 2a), and in metaphases of four *magister* specimens (3♂ and 1♀), a maximum of three NOR-bearing chromosomal pairs were discovered (Nos 3, 9 and 12; Fig. 2b). On the other hand, in the well NOR banded metaphases of two hybrids (2♂), a maximum of four homeologous chromosomes carrying NORs were detected (Nos 3, 7, 9 and 12; Fig. 2c). The average, range and modal numbers of active NORs per cell, counted in the two parental species and their hybrids, are illustrated in Table 2. To compare the relative activity of NORs among parental species and hybrids, we also calculated an index of NOR activity per cell, by dividing the number of active NORs by the number of structural NORs present in each of the analyzed cells. This index was almost equal in the two parental species and their hybrids (Table 2).

As chromosomal pairs carrying NORs in the two parental species karyotypes are homeologous chromosomes, having identical G band patterns (Fig. 1c, 2a and 2b), it was not possible using the NOR-G banding technique, to elucidate the parental origin of each hybrid active NOR-chromosome. Nevertheless, the order of each haploid parental set, according to size and morphology, allowed us to establish that the two parental homeologous chromosomes frequently express their ribosomal genes when both carry structural NORs (pair numbers 3, 9 and 12). However, it was always the case that only one of the homeologous chromosomes number 7 showed active NORs in almost all of the hybrid cells (80 %). This

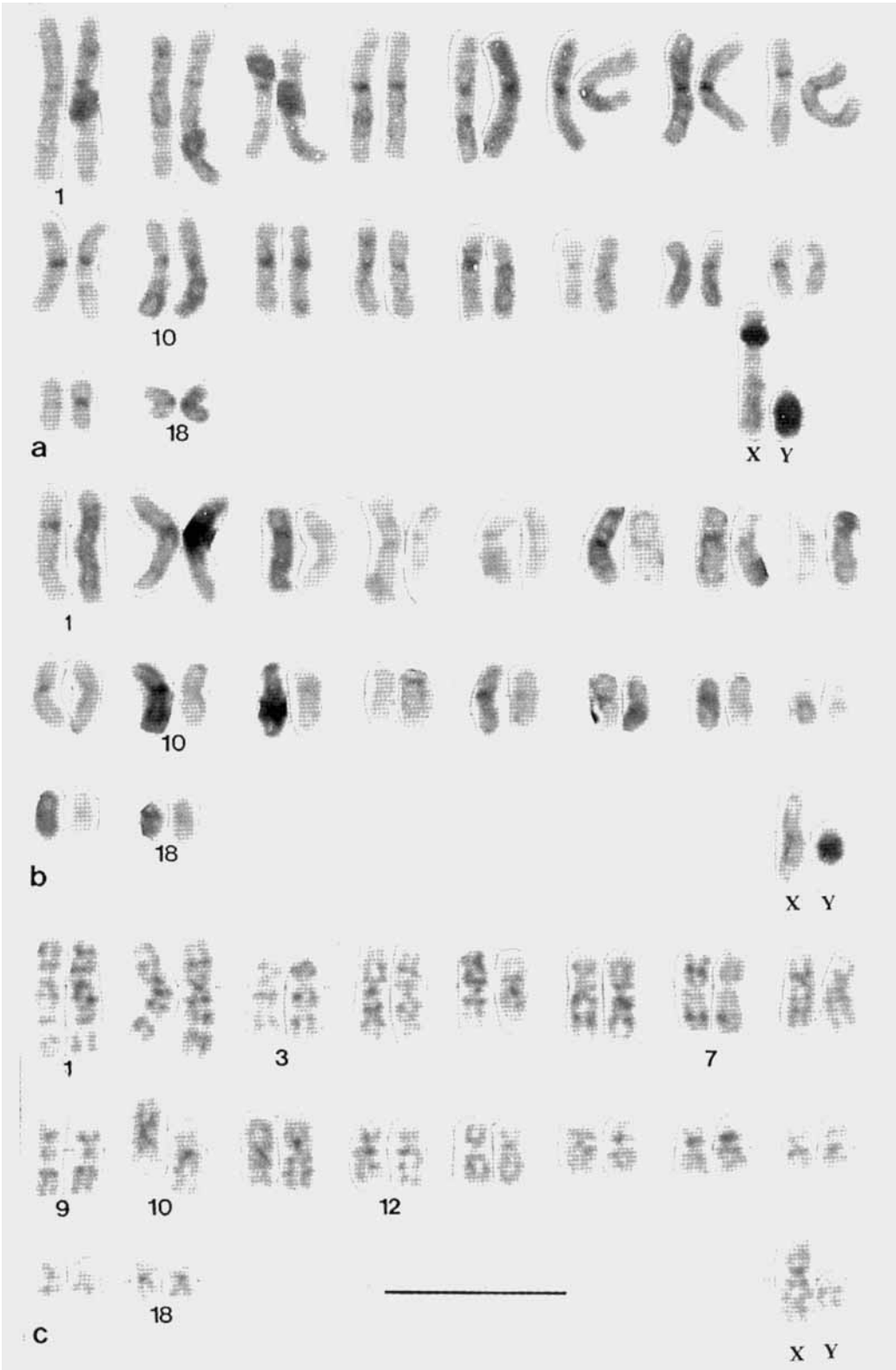

Fig. 1a–c. C-(**a and b**) and G-(**c**) banded karyotypes. **a** *P. magister*, ♂ LCM 1693. **b** ♂ *magister* × ♀ *darwini* hybrid, ♂ LCM 1735. Note that the C bands of *P. magister* and hybrid autosomes and Y chromosomes, are similar. **c** ♂ *magister* × ♀ *darwini* hybrid, ♀ LCM 1740. Differences in the G banding patterns were detected only between one pair of homeologous autosomes (No 10). Sex chromosomes showed the G banding patterns observed in the respective parental species. Chromosomes were ordered and numbered according to a previous description (WALKER et al. 1979). Bar = 10 μm.

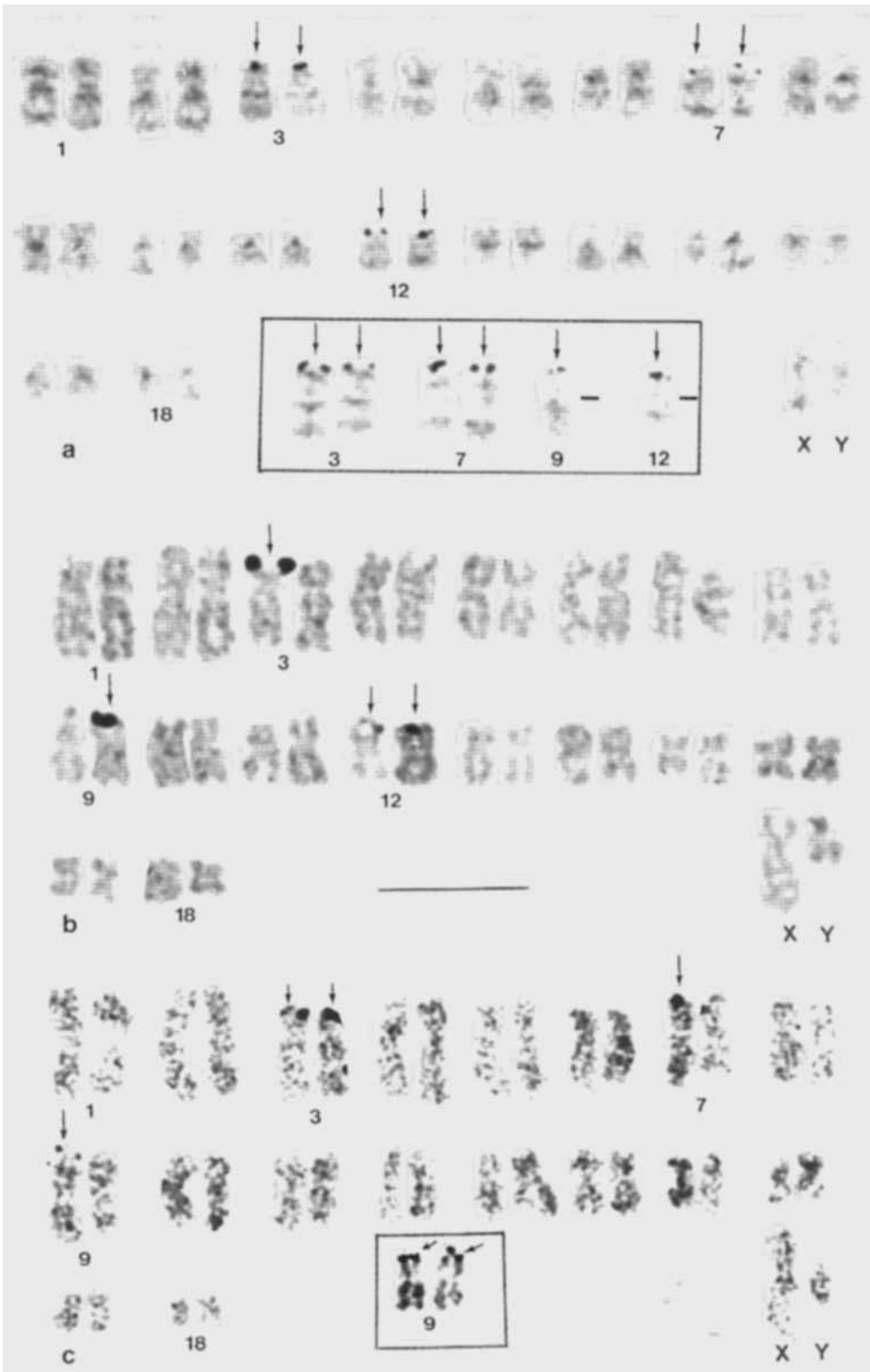

Fig. 2a–c. NOR-G banded karyotypes. **a** *P. darwini*, ♂ LCM 1850. **b** *P. magister*, ♂ LCM 1795. **c** ♂ *magister* × ♀ *darwini* hybrid, ♂ LCM 1740. Arrows indicate the active NOR chromosomes. Insets in (**a**) and (**c**) show the chromosomes having active NORs in other cells of the same animals. Chromosomes were ordered and numbered according to a previous description (WALKER et al. 1979). Bar = 10 μm.

Table 2. *Number of active NORs and index of NOR activity per cell in P. darwini, P. magister and their laboratory hybrids.*

Taxa	n[a]	N[b]	$\bar{X}$	SD	Range	Mode	Index of activity[c]
P. darwini	4	81	3.58	0.77	2–6	4	0.448
P. magister	4	57	3.02	0.77	1–5	3	0.500
Hybrids	2	35	3.14	1.09	1–5	3	0.449

[a] Number of specimens
[b] Number of cells
[c] Mean number of active NORs/number of structural NORs per cell

NOR activity would necessarily correspond to the expression of *P. darwini* rDNA genes, since *P. magister* chromosome 7 does not carry structural NOR (Fig. 2b).

Gonads

Histological sections of *P. darwini* and *P. magister* adult male testes, showed normal germinal lines and interstitial compartments. For a *darwini* adult male (LCM 884), the SI index (GROCOCK and CLARKE 1974) was estimated to be five (Table 3), as the seminiferous tubules were large and had a complete spermatogenic cell line, with abundant spermatids and spermatozoa (Fig. 3a). The ICI index (GROCOCK and CLARKE 1974), was considered to be between four and five (Table 3), as the seminiferous tubules showed large groups of round nuclei interstitial cells (Fig. 3a). The volumetric densities (VD) calculated for seminiferous epithelium, tubular lumen and interstitial compartment of this *darwini* specimen are shown in Table 3.

However, the testes of the two studied hybrids had qualitative and quantitative differences compared to those of the parental taxa and also between themselves (Fig. 3a, 3b and 3c). Thus, the SI index of the LCM 1735 hybrid was estimated to be between zero and one (Table 3), as the seminiferous tubules had a very small diameter and showed a thin epithelium composed of two or three cell layers, which included only spermatogonias, spermatocytes I and Sertoli cells (Fig. 3b). The ICI index of this hybrid was considered to be five (Table 3), since as for the parental *darwini*, it showed many grouped interstitial cells, which in this case, had the appearance of hyperplasic secretory Leydig cells (Fig. 3b). The volumetric densities of seminiferous epithelium, tubular lumen and interstitial compartment, were different from those found in the *darwini* specimen and were also different from the LCM 1740 hybrid. So, the relative volume of epithelium appeared obviously diminished and those corresponding to tubular lumen and interstitial compartment were enlarged, with respect to those detected in *darwini* and the LCM 1740 hybrid (Table 3).

The SI index of the LCM 1740 hybrid was estimated to be between three and four (Table 3), as although some seminiferous tubules had a complete spermatogenic cell line, most of them did not present spermatozoa (Fig. 3c). Moreover, the relative number of elongated spermatids observed, was lower than that showed by all the parental testis sections analyzed (Fig. 3c). Macrophages and picnotic nuclei were found in some tubular lumina. To investigate the destiny of the few detected spermatozoa, we also examined epididyms and vas deferens sections. From a total of 215 epididymae tubules observed, spermatozoa were found in only 15 tubular lumina (Fig. 3d), but they were never seen in vas deferens sections. The ICI index of the LCM 1740 hybrid was considered, as in the LCM 1735 hybrid, to be five (Table 3). The volumetric densities resembled, in general, the VD found in the parental *darwini* specimen more than those detected in the LCM 1735 hybrid, although some significant differences were found (Table 3).

The testis cellular suspensions of two of the hybrids (LCM 1735 and LCM 1964, the single male *darwini* × female *magister* hybrid obtained), showed only spermatogonia, primary spermatocytes with large pachytene-like nuclei and interstitial cells (not shown here), in accordance with the histological study perfomed for these specimens. However, the testis cellular suspension of the LCM 1740 hybrid showed a larger density and diversity of germinal cell types. Thus, besides the interstitial cells, spermatogonia and spermatocytes at different stages of maturation, spermatids and some spermatozoa were also observed (not shown here).

The ovaries of *P. darwini* (LCM 729) and *P. magister* (LCM 1741) adult females exhibited the characteristic appearance of active organs. Many corpora lutea, as well as primordial to mature Graafian follicles, were found (Fig. 4a). The histological appearance of adult hybrid female ovaries (LCM 1739), was similar to that observed in the parental species. Thus, many follicles at different stages of maturation were detected, revealing the existence of a functionally active organ. Nevertheless, no corpora lutea were found in these ovaries (Fig. 4b).

DISCUSSION

Five out of the 13 species which comprise the *Phyllotis* genus can be grouped, according to morphological and molecular characters, into a robust phylogenetic clade named "the *darwini* species group" (STEPPAN 1995, 1998). This clade includes *P. darwini* and *P. magister*, used to obtain the hybrids studied here, and also *P. xanthopygus*, *P. osgoodi* and *P. caprinus* species. The lowest degree of morphological similarity was found between *P. magister* and the other species of the group (STEPPAN 1995); nevertheless, the cladistic analyses of DNA sequences from the mitochondrial cytochrome *b* gene, showed that *magister* is related more closely to *darwini* than to *xanthopygus* (STEPPAN 1998). In accordance with these molecular results, the G and C banded *P. magister* and *P. darwini* karyotypes, share more similarities than they do with any of the other known banded karyotypes of this species group (WALKER et al. 1979, 1984).

The level of genetic difference between the two species can also be inferred from the results of laboratory pairings. Despite morphological and cytogenetical similarities detected between the species, pairings showed a low incidence of success, as measured by the percentage of crosses resulting in births, as well as a high level of aggression within pairs. These results suggest, at the very least, that these species have differences in their behavioural patterns or recognition cues. On the other hand, if we assume that the number of couples and the fertilization rate of interspecific crosses are similar to those of intraspecific crosses, the reduction of births and litter size might be caused by zygotic or embryonic mortality. These losses would then represent a failure of the two genomes to function in harmony.

In mammals, the number of structural NORs per karyotype and their chromosomal distribution, are considered to be species-specific cellular characters. However, NOR transcriptional activity is very variable and it has been proposed that this a characteristic of each individual for many mammalian species (MIKELSAAR et al. 1977; MAYR et al. 1987; SUZUKI et al. 1990; ZURITA et al. 1997). This variability would depend mainly on specific metabolic cell demands, and it has been correlated with different cell types, as well as with sex of the individual (BERRÍOS et al. 1992).

In the *Phyllotis* species studied here, the ribosomal genes were more dispersed in the *darwini* genome, with four chromosomal pairs carrying NORs, than in the *magister* genome, which had only three chromosomal pairs with NORs. Besides this, both species and the hybrids, showed the expected interindividual and intraindividual NOR activity variation, as reflected by the detected standard deviations of NOR averages and by the NOR distributional ranges (Table 2). In the hybrids, with seven chromosomes carrying NORs, the average, range and modal number of active NORs, were more similar to those detected in the parental *magister* karyotype than to those shown by the *darwini* one (Table 2). Although NOR distribution observed in parental and hybrid genomes was different, cells of the three taxa had similar relative NOR activities, as evidenced by their almost equal NOR activity indexes (Table 2), which suggest that certain transcriptional regulator factors would be operating.

Interspecific hybrids have often shown that ribosomal genes of one parental species are transcriptionally dominant over those of the other species. In vertebrates, nucleolar dominance (NAVASHIN 1934) has been detected in hybrids between *Xenopus* amphibian species (CASSIDY and BLACKLER 1974; MACLEOD and BIRD 1982), in hybrids between horse and ass (KOPP et al. 1986; 1988) and also in mouse-human cell hybrids (MILLER et al. 1976). In previous studies, we found that in *Phyllotis darwini* x *P. xanthopygus* hybrids, the activity of *darwini* NORs pre-

Table 3. *Spermatogenic index (SI)*, interstitial cell index (ICI)* and testes volumetric densities (VD) of a parental P. darwini specimen and two different ♂ magister × ♀ darwini hybrids*

	SI	ICI	Volumetric densities (%)		
			Seminiferous epithelium	Tubular lumen	Interstitial compartment
P. darwini (LCM 884)	5	4–5	72.1 ± 4.9	8.0 ± 5.2	19.9 ± 8.0
Hybrid (LCM 1735)	0–1	5	31.5 ± 6.2[a]	41.3 ± 9.6[a]	27.3 ± 8.1[c]
Hybrid (LCM 1740)	3–4	5	64.9 ± 6.9[b]	15.3 ± 8.3[b]	19.9 ± 3.2

*According to GROCOCK and CLARKE (1974)
[a] = $p < 0.001$, respecting *darwini* and the LCM 1740 hybrid
[b] = $p < 0.05$, respecting *darwini*
[c] = $p < 0.05$, respecting the LCM 1740 hybrid

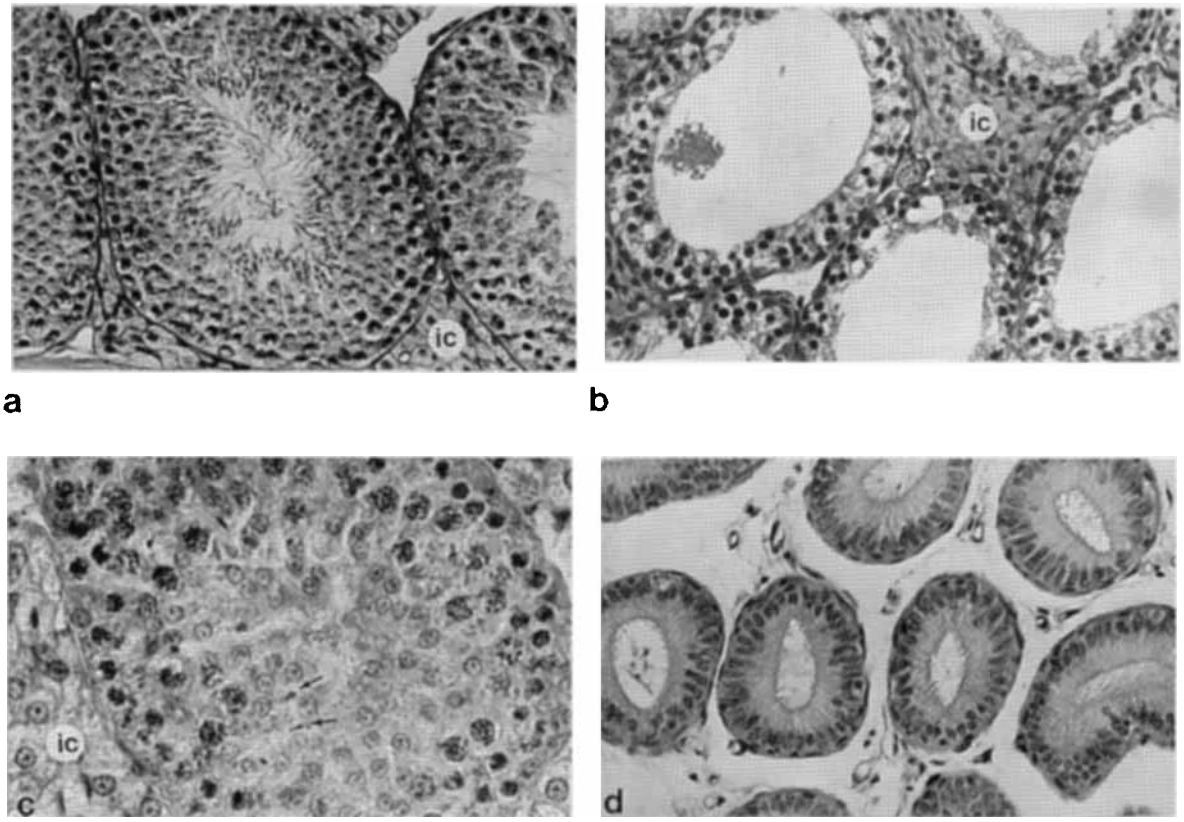

Fig. 3a–d. Testis (**a, b and c**) and epididymis (**d**) sections from parental and hybrid adult specimens. **a** *P. darwini*, ♂ LCM 884 (100 ×); **b** ♂ *magister* × ♀ *darwini* hybrid, ♂ LCM 1735, with no spermatids and spermatozoa, and large groups of interstitial cells (100 ×); **c** ♂ *magister* × ♀ *darwini* hybrid, ♂ LCM 1740, with only a few elongated spermatids (arrows) and no spermatozoa (200 ×); **d** ♂ *magister* × ♀ *darwini* hybrid, ♂ LCM 1740 (100 ×), note the presence of a few spermatozoa in some epididymae tubules. ic = interstitial cells.

vailed over that of *xanthopygus* (WALKER et al. 1998). Nevertheless, for the hybrids studied here, the ribosomal genes of both parental genomes seem to function codominantly.

If this is indeed the case, both NORs located in homeologous chromosomes coming from distinct parental genomes, would be active in the hybrids. Considering the NOR expression variability previously described, we compared the expression frequency of each NOR chromosomal pair, in all hybrid and parental cells (Fig. 5). We found that NOR homeologous chromosomes of hybrids, pairs number 3, 9 and 12, having identical morphology and G bands, presented active NORs in both members of the pair at more or less similar frequencies to those shown by the respective NOR homologous chromosomes of, at least, one parental species. One exception to this trend however, was the NOR located in chromosome 7, which in the hybrids showed a very increased activity (80 %), with respect to that observed in the parental *darwini* species (14.3 %; Fig. 5), from which it is necessarily inherited, as shown by NOR-G band comparisons. Thus, on the whole, this analysis shows that in the hybrids, the ribosomal genes of both parental genomes would be functioning.

Differences in activity of some NOR sites, detected between the two parental genomes and between each parental genomes and the hybrids (Fig. 5), might be considered a consequence of the different number of structural NORs present in any of the karyotypes: eight NOR chromosomes were found in *P. darwini*, six in *P. magister* and seven, in the hybrids. If we assume that the cells of these three forms have similar metabolic demands, then the transcriptional rates of some NORs might be different in the three genomes.

One of the best parameters to evaluate genomic compatibility is meiotic normality and the subsequent gametic differentiation in hybrids. In previously obtained phyllotine hybrids (*P. darwini* x *P. xanthopygus*), males and females were completely sterile. Adults had incomplete germinal lines and lacked differentiated gametes in their gonads (WALKER et al. 1984). In the present case the female *P. darwini* × *P. magister* hybrids can be considered to be fertile individuals because they had normal ovaries, with abun-

dant oocytes contained in follicles at different stages of maturation. On the contrary, hybrid males can be considered to be sterile, because they had testes with total or partial meiotic arrest and absence of or very scarce, gamete production.

Reproductive failure of these hybrids would thus follow Haldane's rule, which states that in animal interspecific hybrids, it is the heterogametic sex that is likely to be absent, infrequent or sterile (HALDANE 1922). In mammals and dipterans with XX: XY sex chromosome systems, it is always the XY male which is affected, whereas in birds and lepidopterans with a ZW: ZZ system, it is always the ZW female.

Many proposals have been formulated to explain Haldane's rule, arguing for either genetic or chromosomal causes. One hypothesis stipulates genetic imbalance or disturbance of the normal epistatic interactions between X chromosomes and autosomes (DOBZHANSKY 1937; COYNE and ORR 1989b), between Y chromosome and autosomes (VIGNEAULT and ZOUROS 1986) or between X and Y chromosomes (COYNE 1985; JONES and BARTON 1985; TUCKER et al. 1992; DOD et al. 1993). Another hypothesis focuses on the abnormal behaviour of the sex chromosomes during hybrid meiosis and gametogenesis, affecting X-Y pairing (KING 1993; RUGARLI et al. 1995) or pachytene XY-autosome associations (FOREJT 1982, 1996; GABRIEL-ROBEZ et al. 1986), which mainly change the conformational and inactivation patterns of the sex chromosomes (JABLONKA and LAMB 1991; KING 1993; FOREJT 1996).

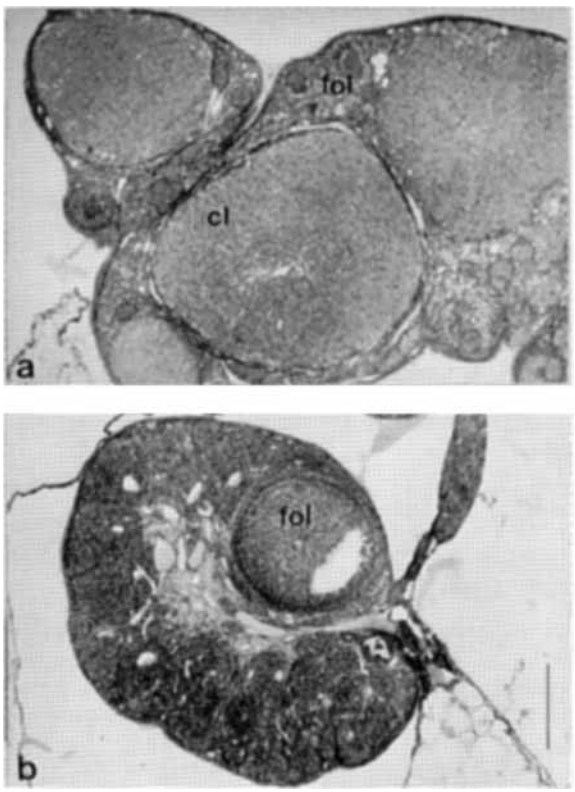

Fig. 4 a and b. Ovarian sections from parental and hybrid adult specimens. **a** *P. darwini*, ♀ LCM 729, with follicles at different maturation stages and various corpora lutea (40 ×). **b** ♂ *magister* × ♀ *darwini* hybrid, ♀ LCM 1739, also showing follicles at different maturation stages but without corpora lutea (40 ×). fol = follicles; cl = corpora lutea.

However, despite much speculation, the genetic basis of Haldane's rule remains still unknown. In a recent review, DAVIES and POMIANKOWSKI (1995) conclude that mutations on the X chromosome must be the basic responsible mechanism: the homozygous sex would be protected by dominant alleles present on the second X chromosome, whereas in the hemizygous sex, all X-linked genes would be phenotypically expressed. However, in mammals there is another mechanism at play, which involves the Y chromosome. Mapping, sequencing and interspecies comparisons of the SRY sex-determining gene, located on the Y chromosome, have shown that the DNA binding domain and presumed functional region of the gene, shows great interspecific variability (TUCKER and LUNDRIGAN 1993; WHITFIELD et al. 1993). The absence of homology between the X chromosome and the Y chromosomal region containing this and other sex-related genes, suppresses recombination and would explain the accumulation of Y chromosome mutations (GRAVES 1995). Thus, the high mutation rates of the Y chromosome would disturb normal interactions between sex-determining Y gene and sex-related X genes and autosomal genes in the hybrids, and would most likely be the main cause of male sterility in mammals (SHORT 1997).

In contrast with most mammalian species studied, phyllotine species show great morphological, G and C banding pattern variability in their sex chromosomes. Moreover, sex chromosomes usually have different morphology or different G and C bands in each phyllotine species, as well as in some subspecies, until now karyotypically described (PEARSON 1972; PEARSON and PATTON 1976; WALKER et al. 1979, 1984, 1991; WALKER and SPOTORNO 1992). *P. darwini* and *P. magister*, with almost identical autosomal sets, differ in the morphology, G and C bands of their X and Y chromosomes (WALKER et al. 1979). So, changes in the sex chromosomes and probably also in sex-linked genes, seems to be the first step in chromosomal and genetic divergence between these species. In their hybrids, chromosomal and genetic interactions between sex chromosomes coming from different genomes, and between sex chromosomes and both parental autosomes, would thus be disturbed, resulting in hybrid male sterility.

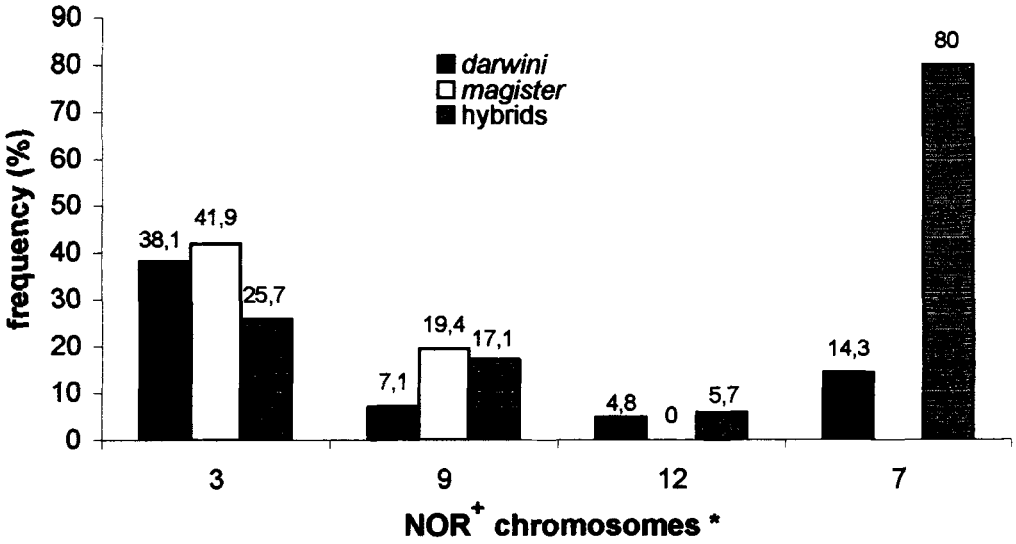

Fig. 5. Frequency of activity showed by the different NOR chromosomal pairs, numbers 3, 9, 12 and 7, in the cells of *P. darwini*, *P. magister* and their hybrids. *Only homologous or homeologous chromosomes with NOR$^+$ in both members of the pair were considered, except for the chromosome 7 of the hybrids.

Haldane's rule is obeyed in all animals known to possess sex chromosomes, which suggests certain fundamental similarities in genetic events causing speciation by postzygotic isolation in these animals. Sterility of the heterogametic sex may be considered to be a first symptom of speciation, because it usually precedes all other reproductive and developmental disharmonies in hybrids (COYNE 1985; ORR 1997). Studies in 119 pairs of *Drosophila* species, which correlate the divergence time, measured by Nei's index of genetic distance (D; NEI 1972), with the strength of reproductive isolation, have shown that the severity of hybrid sterility increases with increased divergence time between parental species (COYNE and ORR 1989a). If the species had recently diverged ($D < 0.5$), they were more weakly isolated and their hybrids almost always conformed to Haldane's rule, compared to those separated a long time ago ($D > 0.5$), which produced sterile male and female hybrids.

Though we do not know the Nei's genetic distance between *P. darwini* and *P. magister*, our data strongly suggest that they have recently diverged. Thus, these chromosomally similar species produced hybrids that conformed to Haldane's rule, and moreover, hybrid males showed variability for the severity of sterility (Table 3). On the other hand, the codominant expression of the ribosomal genes detected in the hybrid cells, indicates that both parental genomes would be functioning in relative harmony and compatibility. In contrast, *P. darwini* and *P. xanthopygus* would have diverged before in time. Thus, both sex hybrids of these cytogenetically different species, were found to be sterile (WALKER et al. 1984), and dominance of *P. darwini* ribosomal genes was found in the hybrid cells (WALKER et al. 1998), suggesting a greater degree of incompatibility between the parental genomes.

At the present time we are performing additional molecular, cytogenetic, reproductive and morphometric studies of these three parental species and their hybrids, in order to analyze the expression of other hybrid phenotypes and the underlying genetic interactions between the parental genomes that control them.

ACKNOWLEDGEMENTS

This work was supported by Proyectos 198 0711 and 195 0628 from the Fondo Nacional de Ciencia y Tecnología (FONDECYT), Chile. We thank Dr. E. Bustos-Obregón for his valuable comments on the manuscript, the Servicio Agrícola y Ganadero, Ministerio de Agricultura, Chile, for granting collection permits, and Juan Oyarce for technical assistance in the collection and care of the animals.

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