Studies on Metatherian Sex Chromosomes. 
IX.* Sex Chromosomes of the Greater Glider 
(Marsupialia : Petauridae)

J. D. Murray, G. M. McKay and G. B. Sharman

School of Biological Sciences, Macquarie University, 
North Ryde, N.S.W. 2113.

Abstract

The greater glider, currently but incorrectly known as *Schinobates volans*, is widely distributed in forested regions in eastern Australia. All animals studied from six different localities had 20 autosomes but there were four chromosomally distinct populations. At Royal National Park, N.S.W., all female greater gliders studied had 22 chromosomes including two large submetacentric X chromosomes with subterminal secondary constrictions in their longer arms. This form of X chromosome occurred also at Bondo State Forest, Myall Lakes and Coff's Harbour, N.S.W., and at Eidsvold, Qld. At Coomoooolaroo, Qld, the X chromosome was also a large submetacentric but a secondary constriction occurred in the shorter arm. Two chromosomally distinct types apparently occur in Royal National Park, one with XY males as in all other populations, and one with XY, Y, males. Y or Y, chromosomes were eliminated from the bone marrow in all populations but were present in spermatogonia, primary spermatocytes and cultured fibroblasts. Animals from Bondo State Forest had three or more acrocentric or metacentric supernumerary chromosomes. 
[Other keywords: C-banding, cytotaxonomy, multiple sex chromosomes, XY bivalent.]

Introduction

Sex chromosome elimination from somatic tissues is relatively rare in mammals. In eight species of marsupial bandicoots, included in the genera *Perameles*, *Isoodon*, *Peroryctes* and *Echymipera*, X chromosome elimination occurs from all cells of some female somatic tissues and Y elimination from all cells of the same male tissues (reviewed by Hayman and Rofe 1977). In two species of *Echymipera* supernumerary chromosomes are lost during development from the same tissues that eliminate a sex chromosome (Hayman et al. 1969; Sharman 1973). Amongst eutherian mammals X chromosome elimination from some female somatic tissues occurs in *Akodon azarae*, *Acomys selousi* and *Chloepus hoffmani*, while somatic elimination of one X chromosome of females and of the single X of male germ cells occurs in *Microtus oregoni* [see White (1973) for review and references]. Y chromosome elimination has been reported to occur in some cells from somatic tissues in man (Pierre and Hoagland 1972) and murine opossum, *Marmosa mitis* (Curcuru-Giordano et al. 1974).

Marsupial multiple sex chromosome systems previously reported have arisen by incorporation of autosomal material into an ancestral XX♀:XY♂ sex determining system. In *Potorous tridactylus* (Sharman and Barber 1952), *Wallabia bicolor* (Sharman 1961) and *Macrotis lagotis* (Martin and Hayman 1967) reciprocal translocations between X chromosomes and acrocentric autosomes resulted in the formation of an XX:XY, Y, system. Hayman and Martin (1965a) showed that portions

of the system of autosomal derivation replicated their DNA synchronously with the remaining autosomes whereas later DNA replication occurred in the nucleolus organizer region of one of the compound X chromosomes of females and in the Y chromosomes of males of *P. tridactylus* and *W. bicolor*. The $X_1X_1X_2X_2 : X_1X_2Y$ system of *Lagorchestes conspicillatus* apparently arose by incorporation of a further autosomal pair into a pre-existing $XX : XY_1Y_2$ system (Martin and Hayman 1966). Amongst eutherian mammals most multiple sex chromosome mechanisms appear to have arisen by X to autosome translocations as in *Sorex araneus* or by Y to autosome translocations as in mongooses [see Fredga (1970) for a review]. However, in the rodent *Vandeleuria o. oleracea* a unique sex chromosome system ($X_1X_1X_2 : X_1X_2Y$) appears to have originated by the dissociation of the original X chromosome into $X_1$ and $X_2$ elements, one chromosome being added to the complements of both male and female (Raman and Sharma 1976). The $XX : XY_1Y_2$ multiple sex determining mechanism of the egg-laying mammal *Tachyglossus* is accompanied in both sexes by six autosomes without obvious homologous partners (Murtagh 1977).

Supernumerary chromosomes are also rare in mammals and in all vertebrates (White 1973). Agar (1923) reported a diploid number of 22 chromosomes and an $XX : XY$ sex determining mechanism in the greater glider (then called *Petauroides volans*). Agar’s specimens presumably came from near Melbourne, Vic., and he did not record the occurrence of supernumerary chromosomes. Hayman and Martin (1965b) confirmed Agar’s results but reported that two to six supernumerary chromosomes were present in a greater glider population from Bondo, N.S.W. Elsewhere amongst marsupials supernumerary chromosomes have been recorded only in the bandicoot genus *Echymipera*. Supernumerary chromosomes have been reported to occur in at least 12 species of eutherian mammals, 11 of which are rodents (Baverstock *et al.* 1976, 1977, and included references).

This paper reports the results of a survey of the chromosomes from six greater glider populations representing three of the four named subspecies of the marsupial presently called *Schoinobates volans* (Kerr, 1792). The generic name is invalid (G. M. McKay, unpublished data) and it is possible that more than one biological species is represented so we will refer to the animals by common name and locality only throughout the paper. We report (1) Y chromosome elimination from the bone marrow in all male animals from all populations studied, (2) the occurrence of $XY_1Y_2$ males in one population, and (3) further data on number and morphology of supernumerary chromosomes in another population.

**Materials and Methods**

Animals collected and collection localities of greater gliders used in this study are given in Table 1. The name first used for the greater glider was *Didelphis volans* Kerr, 1792, and its designated type locality is Sydney, N.S.W. Our material came from Royal National Park, 35 km S. of Sydney. Further collections were made at Bondo State Forest, 25 km E. of Tumut, N.S.W.; Myall Lakes, 100 km NE. of Newcastle, N.S.W., and 28 km N. of Coff’s Harbour, N.S.W. Material from 8·5 km N. of Eidsvold, Qld, is referable to *Petauroides volans incanus* Thomas, 1923, and that from Coomoorboolaroo, 35 km SW. of Duaringa, Qld, to *P. armillatus* Thomas, 1923.

Material for cytological analysis was collected immediately after death from animals shot in the field. Accession numbers of voucher specimens of skins and skulls will be supplied on request to G.M.McK.

Bone marrow was flushed from ends of long bones using Bacto hemagglutination buffer (Difco Laboratories) containing 2·0 µg colchicine ml$^{-1}$, the cell suspension being kept at body temperature for 1½ h to increase the number of metaphase stages of mitosis. After centrifugation at approximately
800 rev/min the supernatant was discarded and the pellet resuspended in 0.53% (w/v) KCl and kept at body temperature for 15 min. After further centrifugation the pellet was fixed in methanol–acetic acid (3 : 1, v/v).

Testis tubes were placed in prewarmed 0.53% (w/v) KCl, shaken vigorously to separate the tubes, kept at body temperature for 30 min and then fixed in methanol–acetic acid (3 : 1, v/v).

Fibroblast cultures were initiated, usually from a piece of ear pinna, following the technique of Murtagh (1977).

Slides from fixed bone marrow and cultured fibroblasts were prepared by dropping a suspension in methanol–acetic acid (3 : 1, v/v) onto cold slides wet with 60% (v/v) acetic acid which were air dried beneath a bench lamp. Testis tubes fixed in methanol–acetic acid (3 : 1, v/v) were centrifuged to pack about 0.15 ml of tubes into the bottom of a 10-ml centrifuge tube. 1.0–1.5 ml of 60% (v/v) acetic acid was added and the opaque suspension which appeared at the top of the acetic acid was drawn into a 2.5-ml disposable syringe fitted with a No. 20 or 21 gauge needle. The material was expressed onto a dry slide on a hot plate at 80°C to form a spreading drop which was immediately drawn back into the syringe so as to leave a film of spread cells on the slide.

Chromosomes were stained in 1% (w/v) lacto aceto orcein and constitutive heterochromatin was stained (C-banded) using the technique of McKenize and Lubs (1973) with the addition of a 2.5-min treatment in a 1:6 (v/v) 0.07 M NaOH–2×SSC (saline sodium citrate) solution following the 0.2 M HCl step.

Results

Chromosome Number and Morphology

The chromosomes of 47 greater gliders (26♀, 21♂) were examined. Twenty autosomes were invariably found—seven pairs of submetacentrics, two pairs ofacrocentrics and one pair of near metacentrics (Fig. 1a). The total chromosome number found varied from 2n = 22 to 2n = 30 but numbers above 2n = 22 were due to the presence of an extra Y chromosome in some males and to the presence of supernumerary chromosomes in one population (Table 1). These aspects are dealt with in succeeding sections.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Sex</th>
<th>No. of animals</th>
<th>2n in somatic cells</th>
<th>No. of bivalents in male meiosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bondo State Forest</td>
<td>♀</td>
<td>1</td>
<td>22±4S</td>
<td></td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>1</td>
<td>22±4S</td>
<td></td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>1</td>
<td>22±4S</td>
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<td></td>
<td>♂</td>
<td>1</td>
<td>22±4S</td>
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<td></td>
<td>♀</td>
<td>1</td>
<td>22±4S</td>
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<td>♀</td>
<td>1</td>
<td>22±4S</td>
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<td>1</td>
<td>22±4S</td>
<td></td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>1</td>
<td>22±4S</td>
<td></td>
</tr>
<tr>
<td>Royal National Park</td>
<td>♀</td>
<td>4</td>
<td>22</td>
<td>11±Y2</td>
</tr>
<tr>
<td>White eye shine</td>
<td>♂</td>
<td>2</td>
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<td>22±3S</td>
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<tr>
<td></td>
<td>♂</td>
<td>2</td>
<td>21+Y2</td>
<td>22+Y2</td>
</tr>
<tr>
<td>Red eye shine</td>
<td>♀</td>
<td>3</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>3</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>3</td>
<td>21±Y2</td>
<td>22</td>
</tr>
<tr>
<td></td>
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<td>3</td>
<td>22</td>
<td>11</td>
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<td>Myall Lakes</td>
<td>♀</td>
<td>5</td>
<td>22</td>
<td>11</td>
</tr>
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<td>♂</td>
<td>6</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>6</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>Coff's Harbour</td>
<td>♀</td>
<td>3</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>3</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>Eidsvold</td>
<td>♀</td>
<td>3</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>3</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>Coomooboolaroo</td>
<td>♀</td>
<td>4</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>2</td>
<td>21</td>
<td>22</td>
</tr>
</tbody>
</table>

A BM = bone marrow, Fibro. = fibroblast cells, S = supernumerary chromosomes.
C-banding regions of apparent constitutive heterochromatin were found at the centromere region of all autosomes (Fig. 2a). All autosomal pairs could be reliably identified in good preparations but when secondary constriction and trabant were not readily recognizable on the X chromosome (see later) there was a possibility of its confusion with the largest, near metacentric, autosome (Fig. 1).

Sex Chromosomes

In all female gliders from Royal National Park 22 chromosomes were invariably found in preparations made from bone marrow or fibroblasts. One pair of large submetacentric chromosomes had secondary constrictions subterminally placed in their longer arms. Since all Royal National Park males had a similar, but unpaired, chromosome these are the X chromosomes (Fig. 1d). This form of X chromosome occurred in all populations except that at Coomooboolaroo (Table 1) where the X is also submetacentric but with the secondary constriction in the shorter arm (Fig. 1c). The longer arm and centromere region of the most common form of X chromosome gave positive C-band staining and there were two C bands in the shorter arm (Fig. 2b). The C-banding pattern in the Coomooboolaroo X chromosome (Fig. 2c) indicates that it apparently differs from the X of other populations by the occurrence of a pericentric inversion involving the central part. However, the changed chromosome is not characteristic of the entire Coomooboolaroo population because one female pouch young had X chromosomes of both types.
In males from all populations studied the Y chromosome was invariably present in spermatogonial mitoses and in cultured fibroblasts from ear or heart tissue (Fig. 3b). It was always absent from bone marrow cells (Fig. 3a). The most common form of Y chromosome is acrocentric and about the same length as the long arm of the X chromosome (Fig. 1d). A second form of Y chromosome, a subacrocentric (Fig. 2b), occurs in the Royal National Park population. In one such Y chromosome a secondary constriction (Fig. 3b) was observed in the short arm in one cell only.

![Fig. 2.](image)

(a) C-banded autosomal karyotype of the greater glider. (b) C-banded preparation of the X, Y₁ and Y₂ chromosomes from a Royal National Park 2n = 23 male. (c) C-banded X chromosomes from the Coomooboolaroo population. Note the presence of the trabant in the long arm of the X chromosomes in (b) and in the short arm in (c).

In two Royal National Park males an additional small acrocentric chromosome (Y₂) was present in spermatogonia, spermatocytes and fibroblasts (Fig. 3d). It was not eliminated from bone marrow (Fig. 3c). The Y₂ chromosome was found in no other population and was restricted to those Royal National Park males which had a white eye shine when observed by spotlight. Red eye shine males and red and white eye shine females lacked the chromosome (Table 1). The limitation of Y₂ to white eye shine males suggests that it is a true Y chromosome.
Both $Y_1$ and $Y_2$ chromosomes of the Royal National Park population are composed largely of heterochromatin although minute areas which do not C band are also present (Fig. 2b).

Meiosis was examined in two males from each population studied. In all cases 10 autosomal bivalents and X and Y chromosomes were identified. At diplotene (Fig. 4a) the X and Y chromosomes (arrowed) pair end to end but the free arm of the X usually folds back giving the impression of Y pairing with both ends of the X. Cells with definite Y pairing with one end only of the X were observed. Locations of X and Y pairing segments in the relevant chromosomes have not been determined. Pairing of X and Y also occurs by end-to-end association in the Coomooboolaroo population (Fig. 4d). We are unable to determine whether crossing-over between X and Y takes place or whether meiotic segregation occurs in the absence of chiasma formation.
Pairing between X and $Y_1$ was normal in the Royal National Park $2n = 23$ white eye shine males but the $Y_2$ chromosome was associated with X and $Y_1$ in only 37% of cells examined (Table 2). When associated the $Y_2$ was paired at the same end of the X as was the $Y_1$. In most cells X and $Y_1$ formed a bivalent while the $Y_2$ chromosome was close by, but not actually paired (Fig. 4b). However, in 10% of all cells examined neither Y was paired with the X chromosome but in no case did the two Y chromosomes pair together when the X was not associated.

![Cells during diplotene of male meiosis.](image-url)
Supernumerary Chromosomes

Extra chromosomes, other than the Y2 of the Royal National Park white eye shine males, were found only in the Bondo population (Table 1). In six individuals between three and eight supernumeraries were observed. They were present in bone marrow, cultured fibroblasts and during spermatogenesis and (so far as could be ascertained) were constant in number from cell to cell of the same individual. Both acrocentric and metacentric supernumeraries were observed in cells from most individuals (Fig. 1b).

Table 2. Numbers of associations of the XY bivalent with Y2 or supernumerary chromosomes during diplotene of male meiosis

<table>
<thead>
<tr>
<th>Y2 in Royal National Park XY1Y2 type</th>
<th>Supernumerary chromosomes in Bondo State Forest population</th>
</tr>
</thead>
<tbody>
<tr>
<td>With XY</td>
<td>22</td>
</tr>
<tr>
<td>Not with XY</td>
<td>38</td>
</tr>
<tr>
<td>With supernummary</td>
<td>N/A</td>
</tr>
<tr>
<td>Total cells scored</td>
<td>60</td>
</tr>
</tbody>
</table>

^A One supernumerary bivalent was additionally associated with the XY bivalent, so total does not add to 45.

At diplotene of male meiosis the supernumeraries were usually present as univalents although occasional bivalent associations or association with the XY bivalent were observed (Fig. 4c). Fifteen diplotene cells from an individual with three supernumerary chromosomes were analysed. In 11 of these the supernumeraries occurred as univalents and were not associated with any bivalent. In a further two cells one and two supernumeraries were joined to the XY bivalent by thin chromatin threads—in the first case to the XY pairing region and in the second to the centromeric region of the X. In yet another two cells the supernumeraries were present as univalent and bivalent and in one of these the bivalent was connected to the XY pairing region by thin threads (Table 2).

Discussion

The results confirm that the basic diploid chromosome number in the greater glider is 22, with an XX ♀: XY ♂ sex determining system as previously reported (Agar 1923; Hayman and Martin 1965b). However, the Y chromosome was not correctly identified by these workers. The autosomal karyotype was found to be invariant among the 47 animals studied, but three different variations in the sex chromosomes were observed. These include (1) an X chromosome inversion in the Coomooboolaroo population; (2) the occurrence of Y chromosome elimination from the bone marrow of adult males, and (3) the addition of the Y2 element with a corresponding increase in chromosome number to 23 in white eye shine males from the Royal National Park population.

Y Chromosome Elimination

The most interesting observation was the occurrence of a system of Y chromosome elimination from the bone marrow of adult males. This phenomenon occurred in all
bone marrow cells from all males examined, including the $2n = 23$ individuals, which indicates that it is a general feature in all greater gliders. A strictly analogous system has not yet been reported for any other mammal.

Y chromosome elimination from the bone marrow of man (Pierre and Hoagland 1972) or fibroblasts of *Marmosa mitis* (Curcuru-Giordano et al. 1974) involves only a proportion of the cells in the tissue and, at least for man, the elimination appears to be a function of ageing (Pierre and Hoagland 1972). The system in *M. mitis* would appear to be unrelated as Y chromosome loss was observed in 14 out of 129 cells in cultured fibroblasts while in the greater glider elimination from ear- and heart-derived fibroblasts did not occur.

The Y chromosome elimination system in the greater glider is most similar to the X and Y chromosome elimination system found in bandicoots. In bandicoots, bone marrow, spleen, gut and cornea are known to eliminate an X chromosome in females and a Y chromosome in males (Hayman and Martin 1965c; Walton 1971) while skin-derived fibroblasts do not eliminate a sex chromosome (Jackson and Ellem 1968; Close 1975; C. E. Murtagh, personal communication). In addition, the supernumerary chromosomes found in bandicoots of the genus *Echymipera* are also eliminated from all somatic tissues which eliminate a sex chromosome (Hayman et al. 1969; Sharman 1973). The system in the greater glider is distinctly different although similar tissues are involved. The X chromosome is not eliminated from bone marrow in females and the supernumerary chromosomes are not eliminated from bone marrow cells in either sex. In a marsupial hybrid, *Macropus giganteus* $\delta \times M. rufogriseus$, 89% of cultured leucocytes lacked a Y chromosome (Sharman, unpublished data) but the Y chromosome was present in most, if not all, cells in fibroblast cultures from the same hybrid (D. W. Cooper, personal communication). Y chromosome elimination from all cells of a somatic tissue, such as occurs in male bandicoots, has not been reported amongst eutherian mammals.

The data of Walton (1971) and Hayman and Martin (1974) indicate a developmental sequence of timing of sex chromosome elimination in *Perameles*. Close (1975) has shown that certain liver cells become XO early in pouch life followed by spleen cells and then bone marrow cells, thus clearly illustrating that elimination occurs in different tissues at different times during development. Y chromosome elimination from liver, spleen and bone marrow cells during the pouch life of the greater glider is discussed in another paper (Murray and McKay 1979).

Hayman and Martin (1965c), Walton (1971) and Hayman (1977) suggested that X chromosome elimination in bandicoots is an extension of the X chromosome inactivation or dosage compensation system which occurs in mammals (Lyon 1961). Bianchi and Contreras (1967) have also suggested that this as an explanation for X chromosome or partial X chromosome elimination in *Akodon azarae*. However, Y chromosome elimination in the greater glider cannot be explained in terms of dosage compensation unless it is assumed that the Y carries some genetic information (e.g. former X-linked genes) which require inactivation to maintain the correct genetic balance. At present there is no information to support this suggestion in either greater gliders or bandicoots. However, it is perhaps significant that fibroblast cells, which retain Y chromosomes in greater gliders and bandicoots, also express partial activity of the normally inactive paternal X chromosome in kangaroos (Cooper et al. 1977; Johnston et al. 1978).
Y₂ Chromosome

In two male greater gliders from Royal National Park the addition of the Y₂ chromosome increases the diploid number to 22 in bone marrow and 23 in fibroblast cells and germ line tissues (Table 1). Although it appears to be meiotically unstable the Y₂ does not behave in the totally random manner exhibited by the supernumerary chromosomes of the Bondo State Forest population. It associates only with the pairing end of the X chromosome, unlike the supernumeraries where two out of five associations occurred with the centromere of the X chromosome. It thus appears unlikely that the Y₂ is simply a single supernumerary chromosome.

The Y₁ and Y₂ chromosomes of the Royal National Park white eye shine type may have originated by dissociation of an ancestral Y chromosome with subsequent increase in constitutive heterochromatin to bring Y₁ to the same size as the Y chromosome of other glider populations. Dissociation of a chromosome without donation of a new centromere is an unlikely event (White 1973) so a reciprocal translocation with a pre-existing supernumerary chromosome may have occurred. This explanation relies on the prior occurrence of supernumerary chromosomes in the ancestors of the Royal National Park white eye shine animals and assumes that they have been subsequently lost from that population.

The addition of a second Y chromosome in the greater glider is similar to the reported addition of a second X chromosome in the mouse Vandeleuria o. oleracea (Raman and Sharma 1976). However, in Vandeleuria Raman and Sharma have shown that the X₁+X₂ would equal approximately 5% of the genome, the size of the postulated conservative mammalian X chromosome (Ohno 1967). A similar argument for the dissociation event cannot be made for the greater glider Y chromosome.

Supernumerary Chromosomes

Hayman and Martin (1965b) reported between two and six supernumerary chromosomes in the Bondo State Forest greater glider population. An additional six individuals from this population examined by ourselves had between three and eight supernumeraries. However, supernumerary chromosomes were not found in the other five populations of greater gliders examined, nor were they reported by Agar (1923).

The supernumerary chromosomes of the greater glider were found to be entirely C-band positive. This agrees with the reports that the supernumeraries of the rodents Uromys caudimaculatus, Mastacomys fuscus (Baverstock et al. 1976, 1977) and Rattus rattus (Yosida and Sagai 1975) are composed of C-band positive material.

Cytotaxonomy

At present all populations of greater gliders are considered to be conspecific. The results of this survey indicate that cytogenetically the gliders may be divided into four groups: (1) Bondo State Forest—supernumeraries; (2) Coomooboolaroo—inverted X chromosome; (3) Royal National Park—2n = 23, Y₂ chromosome; and (4) the remaining 2n = 22, XY populations. While it is unlikely that any of these differences would lead directly to speciation it is possible that some of them may contribute in some degree to a separation of the respective gene pools. At present morphometric data (McKay and Murray, unpublished data) indicate that both the Bondo State Forest and Coomooboolaroo populations may be unique. However,
on morphometric criteria the $2n = 22$ and $2n = 23$ types from Royal National Park are not significantly different, even though they differ by one chromosome in the males.

Acknowledgments

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