The chromosomes of the southern hemispheric lamprey, *Geotria australis* Gray[1]

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Summary. Approximately 180 small and mainly acrocentric chromosomes are present in *Geotria australis* (Geotriidae) from the southern hemisphere. This is closer to the situation found in northern hemispheric species (Petromyzonidae) than in other southern hemispheric lampreys (Mordaciidae).

The living lampreys (Petromyzoniformes) have an antitropical distribution[2]. The majority of the 39 species are located in the northern hemisphere and these are now generally regarded as comprising a sufficiently homogeneous group to justify their assignment to a single family, the Petromyzonidae[3,4]. By contrast, there has been disagreement regarding the systematic status of the 2 southern hemispheric genera *Mordacia* and *Geotria*[5], although the more recent analyses of various criteria suggest that both merit separate familial distinction (Mordaciidae and Geotriidae)[2,3]. Each of the 3 species of *Mordacia* is restricted to either South America or Australia, whereas the monotypic *Geotria* is present in both these continents and in New Zealand[2,3,5].

Studies on the chromosomes of several petromyzonid species have shown that their numbers are markedly higher (164–168)[6–9] than those of *Mordacia* spp. (76)[10,11]. The current study on the chromosomes of *Geotria australis* was undertaken to permit cytotaxonomic comparisons between the 3 extant lamprey families.

Ammocoetes of *Geotria australis* were collected with an electric fish shocker from various tributaries of the Warren and Donnelly Rivers in south-western Australia. The ammocoetes were placed in 0.1% colchicine for the 12 h immediately prior to sacrifice. Air-dried preparations of gill material, stained with 2% aceto-orcein, were made after the method of Howell and Denton[6]. Adults were collected with dip-nets at the face of a weir on a major tributary of the Warren River soon after they had embarked on their upstream migration from the sea. Air-dried preparations of testes were made using the method described for mammalian material by Luciani et al.[12].

Determination of precise diploid numbers in lampreys from mitotic metaphase spreads presents a variety of problems. The mitotic index in somatic tissues is low and it is extremely difficult to prepare complete complements in which exceptionally high numbers of very small chromosomes are adequately separated. These problems were accentuated in *G. australis* and the spreads examined from gill tissue of a large number of healthy ammocoetes were inferior to most of those that have been obtained using the same techniques on other species of lampreys. However, 11 counts of between 170 and 190 were recorded for *G. australis* and 8 of these were between 174 and 184. Counts below 170 varied widely and were assumed to be the result of preparative loss. A regular feature of the spreads was that 8–10 of the chromosomes were distinctly larger than the remainder of the set, a feature previously observed in the karyotypes of petromyzonids[9]. Several of the larger group are apparently meta- or submetacentric, whereas many of the smaller chromosomes appear to be acrocentric. Although the absence of a sharply defined modal diploid number might reflect variation due to differences in such features as the number of micro- or supernumery chromosomes, it seems more likely to have stemmed predominantly from the problems of obtaining precise counts.

Meiotic configurations from spermatocytes in prophase I were examined in preparations made from the adults migrating towards their spawning grounds. Many bivalents at early and middle prophase showed conspicuous segments of precociously-condensed chromatin (figure 1) at one or both ends as well as interstitially. These heterochromatic regions often obscured the precise limits of individual bivalents in these stages (which included most of the testicular material present), but in a few spreads of cells in late prophase I (figure 2) bivalent counts of about 90 were obtained.

The chromosomal complement of the sole representative of the Geotriidae contrasts markedly with those of the Mordaciidae in which the diploid number is 76 and the chromosomes are mainly or entirely meta- or submetacentric[10,11]. The karyotype of *G. australis* is, in fact, more similar to those of the northern hemispheric lampreys, although its chromosomal number is greater than the consistent values of 164–168 recorded for eight different petromyzonid species[9]. Such data can be equated with views on the evolution of the groups of living lampreys. For example, on the basis of morphology, *Geotria* and *Mordacia* are not considered to be closely related, even though it has been suggested that each may have arisen from a stock which possessed features similar to those of the more primitive holarctic lampreys[2,3,5]. Since *Geotria* shares some morphological characters with the northern hemispheric lampreys and some with *Mordacia*, it would seem reasonable to assume that the divergence of *Mordacia* occurred earlier than that of *Geo-*

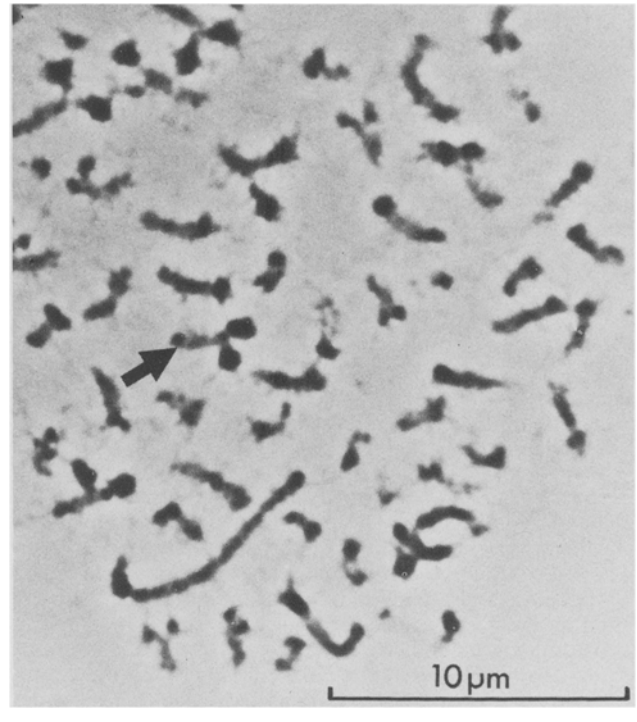

Fig. 1. Part of a meiotic (pachytene) spread from a spermatocyte showing terminally-located blocks of heterochromatin on each homologue (arrow indicates an example).

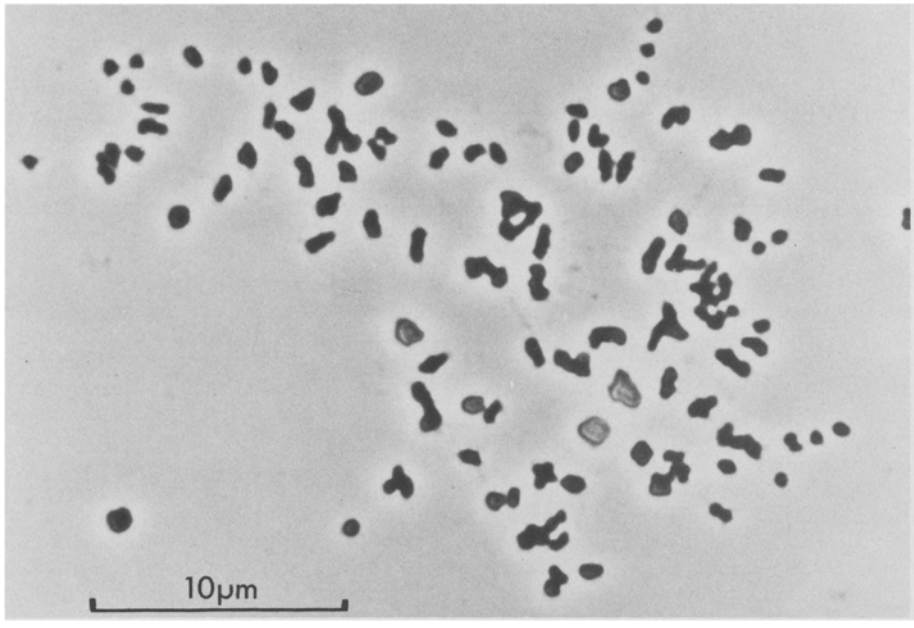

Fig. 2. Spermatocyte diakinesis with about 90 bivalents present.

tria. Such trends would be consistent with the modification of the *Mordacia* karyotype, including the reduction in chromosome number through such changes as centric fusions[9,13], and the possession of a large number of chromosomes in *Geotria.*

The information on the karyotype of *G. australis* presented in this paper suggests that this southern hemispheric species has the highest diploid number so far recorded for any vertebrate.

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Differential staining of a heterochromatic zone in *Arcyptera fusca* (Orthoptera)[1]

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Summary. A homogeneous heterochromatic zone obtained with the direct application of the C-banding technique is unravelled into 2 sub-zones with the successive application of orcein staining and the C-banding method in the grasshopper *Arcyptera fusca.*

It has been argued that heterochromatin is the most dynamic of all chromosome components and although it has been intensively studied during the last years we still know too little about this component of the genome. The C-banding[2] and the N-banding techniques[3] are actually used as methods which offer a simple means of defining constitutively heterochromatic regions within the chromosome. However, there exist differences between the C and the heterochromatin N-banding patterns[4,5] within the same individual, which means that 2 different banding methods may reveal a specific differentiation within constitutive heterochromatin.

In this note, we report a singular case where a positive C-band, placed in the terminal region of a short autosome in the grasshopper *Arcyptera fusca* (Orthoptera: Acrididae), is unfolded with the combined action of both conventional orcein staining and the C-banding method. Testes of *A. fusca* are fixed in 3:1 ethanol: acetic acid for 24 h and stored in 70% ethanol. Squash preparations are made in lactopropionic orcein and frozen in liquid nitrogen. The coverslip is removed with a razor blade and left 1–2 days before proceeding further. Slides are steeped in a Coplin jar of freshly prepared 5% aqueous solution of barium hydroxide at 60 °C for 30 min, then rinsed thoroughly with