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KARYOTYPIC EVOLUTION IN PLEUROCERID SNAILS
II. *PLEUROCERA, GONIOBASIS,* AND *JUGA*

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ABSTRACT

Rather little variation was noted among the karyotypes of three species of *Pleurocera,* seven species of *Goniobasis,* and one species of *Juga.* Most species have diploid numbers of 34, the remainder show 36. *Pleurocera unciale* seems to differ from *Goniobasis simplex* by but a single pericentric inversion, while the karyotypes of *Juga hemphilli* and *Goniobasis livescens* are indistinguishable. There is some evidence that the most ancestral karyotype belongs to one of these four species. Greater variation was noted among species within *Goniobasis* and *Pleurocera,* with numerous inversions and occasional translocations apparent. Most species of *Goniobasis* seem to be equally related to several others in the genus, implying a uniform time of origin.

Key words: snails, freshwater, Pleuroceridae, cytogenetics, chromosomes, karyotypes, evolution.

INTRODUCTION

In the first paper of this series, I pointed out that although freshwater snails of the family Pleuroceridae have figured prominently in many evolutionary and ecological studies, their systematics are poorly understood (Dillon, 1989). I reviewed data from several previous studies (Patterson, 1969; Chambers, 1982; Dillon, 1982) suggesting that pleurocerid snails may be karyotypically variable and proposed a survey of karyotypic variation over all six genera. I then used flow cytometry to demonstrate that total genomic DNA is constant in pleurocerids, a result suggesting karyotypic conservation, at least in regard to such phenomena as large-scale gene duplication.

This would not preclude the occurrence of Robertsonian fusions or fissions, inversions, translocations, or any other such structural changes as might be useful in reconstructing the evolutionary history of a group of organisms. White (1973, 1978) has reviewed many very successful applications of cytogenetics to these purposes, not only in the polytene dipterans such as fruit flies, midges, and mosquitos, but in other insects, plants, amphibians, reptiles, and mammals. Among freshwater snails, most attention has focused on the pulmonates (Patterson & Burch, 1978), especially medically important planorbids (Goldman et al., 1983, 1984). Recently, Nakamura & Ojima (1990) have added data on cellular DNA content to the already extensive information on karyotypic evolution in Japanese *Semisulcospira* (Burch, 1968).

Three of the six pleurocerid genera, *Pleurocera, Goniobasis,* and *Juga,* are characterized by shell lengths much greater than shell width. Although few members of this family are characterized by preference for soft substrates, these three genera do not seem as restricted to rocky bottoms as do *Io, Lithasia,* and *Leptoxis. Pleurocera, Goniobasis,* and *Juga* would thus seem to constitute a natural subgroup of pleurocerids, and their karyotypic morphology is the subject of this investigation.

METHODS

The karyotypes of the following ten species are newly reported here: *Goniobasis acutocarinata* (Lea) from southwestern Virginia, *G. alabamensis* (Lea) from Alabama, *G. catenaria dislocata* (Reeve) from South Carolina, *G. livescens* (Menke) from Michigan, *G. proxima* (Say) from North Carolina, *G. simplex* (Say) from southwestern Virginia, *Juga hemphilli* (Henderson) from Oregon, *Pleurocera acuta* Rafinesque from Michigan, *P. canaliculatum* (Say) from Tennessee, and *P. unciale* (Reeve) from Tennessee. Photographs and full locality data are given in Dillon (1989). In addition, I have included in this analysis the karyotype of *Goniobasis floridensis* (Reeve) as published by Chambers (1982), taken from an original photograph kindly provided by the author.

The primary challenge of this investigation was to obtain preparations with sufficient concentrations of cells at mitotic metaphase. Striking differences between individual snails were noticed in this regard, as well as short-term temporal and seasonal differences (summer collections being best). Snails were generally held in their native water at environmental temperature and treated as soon as possible after collection. Preparations were generally made from a large number of males (20–40) and preliminarily screened to identify five from which karyotypic data could most easily be obtained.

Snails were transferred to beakers of native water (usually about 10–20 individuals in 200 ml), with a pinpoint bubbler for aeration, and allowed to come to room temperature gradually. Colchicine was added directly to the water at a concentration of 0.5 mg/ml (0.05%, w/v). Later in the study, it was discovered that the addition of a lectin, "pokeweed mitogen" (Sigma L-9379), effectively increased the mitotic index at a concentration of 10 μg/ml. Snails were removed from this solution at intervals from 12 to 24 hours, with about 18 hours usually providing best results.

The cytogenetic techniques employed here are an original modification of standard clinical methods. Snails were washed, cracked with pliers, and females and parasitized individuals discarded. The testes were dissected from healthy males and placed in about 1–2 ml of distilled water in a clear plastic culture tube. (Initial experiments showed that standard hypotonic treatments, such as 0.075 M KCl, had little effect on these cells.) The tissue was disrupted by drawing the entire volume in and out of a Pasteur pipette rapidly and repeatedly. The sample was then incubated at 37°C for 30 minutes.

The sample was acidified with a drop of fixative and gently centrifuged (5 minutes at about 500 rpm on a large, swinging-bucket rotor). The supernatant was aspirated and the pellet resuspended in freshly prepared modified Carnoy's fixative (3 methanol:1 glacial acetic acid). To prevent clumping, the pellet was agitated with a pinpoint bubbler as the fixative was added dropwise. After 15 minutes, the sample was recentrifuged and resuspended in fresh fixative. It was found that cells fixed in this fashion could be held under refrigeration for at least two weeks.

Clean slides were kept in distilled water at 3°C. Cells in fresh fixative were dropped onto these slides from a distance of about an arm's length, treated briefly with steam, and dried at 55°C. Then a syringe was used to layer fresh Wright's stain over each slide for 20–60 seconds. The stain was a dilution of 1 part Wright's stock (0.25% in methanol) to 4 parts phosphate buffer (0.025 M Na_2HPO_4, 0.025 M KH_2PO_4, pH 6.86). Slides were washed in water, blown dry, dipped in xylene and mounted in Permount (Fisher).

Early in this study, I experimented with a number of chromosome banding techniques, including the "ASG" and "BSG" methods (Sumner et al., 1971; Sumner, 1972) and trypsin banding (Seabright, 1971). But the fairly lengthy colchicine treatment required to obtain a reasonable mitotic index had the undesirable side-effect of yielding rather condensed chromosomes. Thus banding was not reliably induced, and this approach was discontinued.

Idiograms were constructed in standard fashion, clipping and measuring photographs. The summed length of all chromosomes was held constant, given the findings of Dillon (1989). Chromosomes were assigned a qualitative size category: "small" if representing less than 5% of the haploid genome, "medium" if 5–10%, and "large" if over 10%. Each chromosome was also assigned a category following Levan et al. (1964): metacentrics showing an arm-length ratio of 1.0–1.7, submetacentrics 1.7–3.0, acrocentrics 3.0–7.0, and telocentrics greater than 7.0. Chromosomes in typical preparations ranged only from about 2–6 μm, rendering measurements of arm-length ratio approximate in many cases.

For convenience I have considered any change in arm-length ratio as due to a pericentric inversion, although certain translocations could also account for a shift in centromeric position. It should be emphasized that only a small proportion of all structural variation is detectable by these techniques. Each karyotype was compared to that of all other species to identify similarities, and a hypothesis formed to account for observed variation assuming minimum rearrangement, as has become standard for studies of this sort (White, 1973, 1978).

RESULTS AND DISCUSSION

The example karyotypes from the three genera shown in Figure 1 are strikingly similar. *Goniobasis simplex* and *Pleurocera un-*

FIG. 1. Exmaple karyotypes for three genera of pleurocerids. For each preparation, chromosomes are sorted by size in three categories: metacentric (M), submetacentric (S), and acrocentric (A).

ciale both have diploid numbers of 34, and seem to differ by as little as a single pericentric inversion in one of their medium-sized chromosomes. *Juga* has a diploid number of 36, differing from *G. simplex* only by an apparent centric fusion/fission event involving a medium-sized metacentric and two small acrocentric chromosomes.

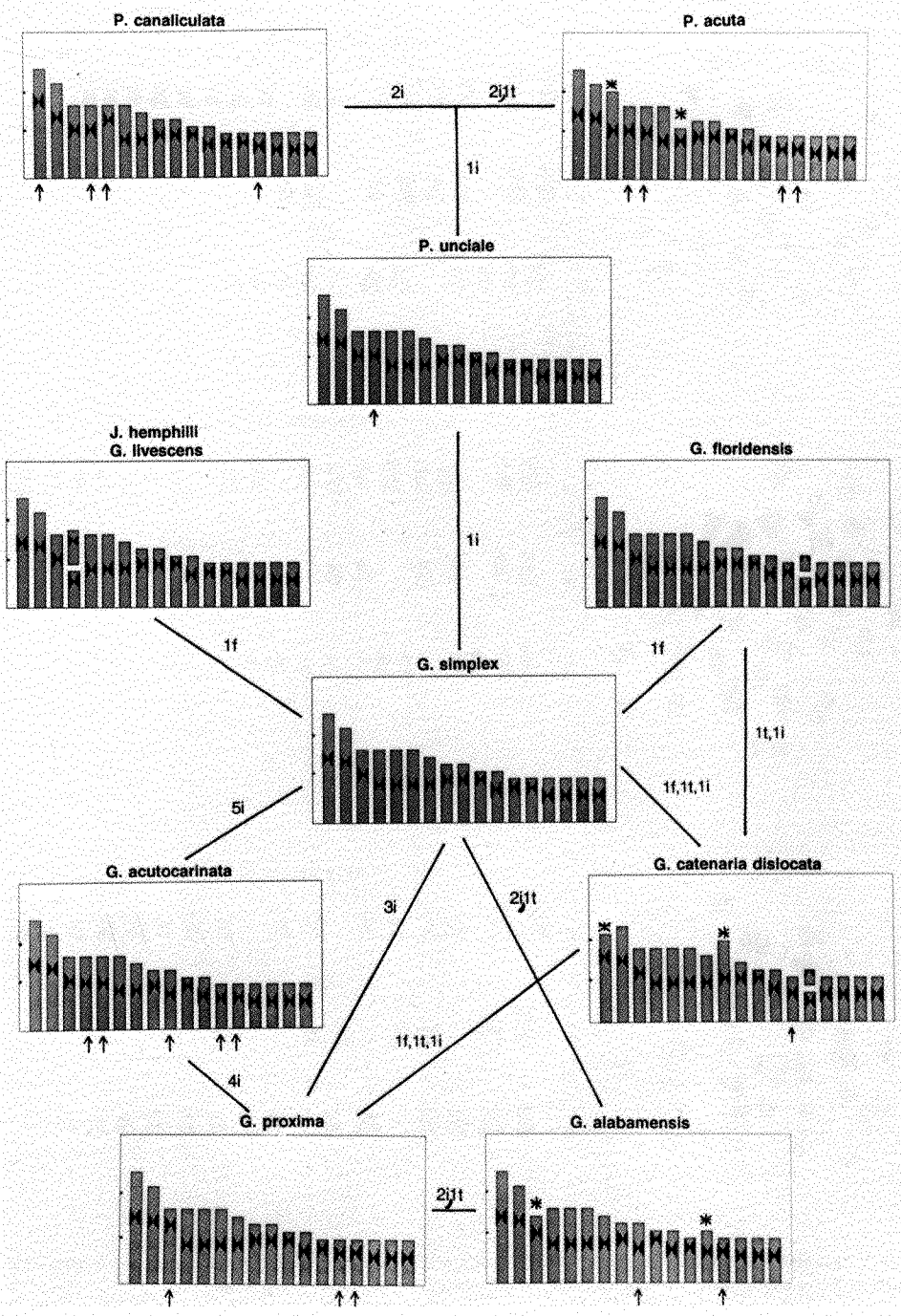

FIG. 2. Idiograms for the 11 pleurocerid species treated in this study, together with their hypothesized relationships. Centromeric positions are approximate, especially in the smaller chromosomes. Ticks on the ordinate mark 5% and 10% of the haploid genome. Arrows show inversions (i) and stars show translocations (t) relative to the karyotype of *Goniobasis simplex*, the standard. Centric fusion/fission events are designated f.

Idiograms for all 11 species are displayed in Figure 2, linked together with karyotypically similar species. Karyotypes are characterized by chromosome size and centromeric position in Tables 1 and 2. Only *Juga, G. livescens* (karyotypically indistinguishable from *Juga*), *G. catenaria dislocata,* and *G. floridensis* (as previously reported) have 2N = 36. The remainder of the taxa show 2N = 34. The *G. simplex* karyotype was designated as archetypical or "standard," because of its central position in Figure 2. (Coincidentally, this species also served as the standard in the isozyme study of Dillon & Davis, 1980.) Arrows locate apparent pericentric inversions and stars locate reciprocal translocations, relative to *G. simplex.*

Given previous reports of karyotypic variation in the Pleuroceridae, this degree of conservation was unexpected. Chambers (1982) reported that two populations of *G. floridensis* from the Florida panhandle differed from each other by at least one chromosomal rearrangement, and differed from the peninsular karyotype shown in Figure 2 by a minimum of three rearrangements. Here it is reported that no greater difference is apparent between pleurocerid genera. But the degree of structural variation in karyotype is clearly underestimated by the techniques employed here. Chambers (1987) has argued that snails are generally not much more karyotypically conservative than other animals, including the mammals. The apparently greater rates of chromosomal evolution that have been attributed to mammals may be a function of their geological youth.

Although the relationship among the *Pleurocera* species shown in Figure 2 is rather "treelike," relationships among most of the *Goniobasis* species are unresolved. The karyotypes of *G. acutocarinata, G. alabamensis, G. catenaria dislocata,* and *G. proxima* each contain distinctive elements while remaining as similar to *G. simplex* as to any other taxon. All five of these *Goniobasis* species could plausibly be related to at least two other taxa. This suggests both that these species of *Goniobasis* may have diverged at about the same time, and that some of the approximately 80 *Goniobasis* species unanalyzed may have karyotypes intermediate between these five.

It should also be noted that implicit in Figure 2 is the assumption that all chromosome rearrangements are equally diagnostic. But the pericentric inversions in particular are difficult to score, and may not contain as much phylogenetic information as translocations or fusion/fission events. Thus one might reasonably hypothesize, for example, that *G. flori-*

TABLE 1. *Goniobasis* karyotypes, categorized by the criteria described in text. M—metacentric, SM—submetacentric, A—acrocentric, T—telocentric, Lg—large, Md—medium, Sm—small.

Species	Chrom Size	Centromeric Position M	SM	A	T
G. acutocarinata	Lg	1	1		
	Md	3	3	1	
	Sm	4	3	1	
G. alabamensis	Lg	1	1		
	Md	4	2	1	
	Sm	5	1	2	
G. cat. dislocata	Lg	1	2		
	Md	4	1	1	
	Sm	5	2	1	1
G. floridensis	Lg	1	1		
	Md	4	1	2	
	Sm	4	2	2	1
G. livescens	Lg	1	1		
	Md	2	1	3	
	Sm	5	1	4	
G. proxima	Lg	1	1		
	Md	3	1	3	
	Sm	3	3	2	
G. simplex	Lg	1	1		
	Md	4	1	2	
	Sm	4	1	3	

TABLE 2. The karyotypes of *Pleurocera* and *Juga,* categorized by the criteria described in the text. M—metacentric, SM—submetacentric, A—acrocentric, Lg—large, Md—medium, Sm—small.

Species	Chrom. Size	Cent. Position M	SM	A
P. acuta	Lg	2	1	
	Md	1	2	2
	Sm	3	3	3
P. canaliculata	Lg		2	
	Md	2	2	3
	Sm	3	2	3
P. unciale	Lg	1	1	
	Md	3	2	2
	Sm	4	1	3
J. hemphilli	Lg	1	1	
	Md	2	1	3
	Sm	5	1	4

densis and *G. catenaria dislocata* share a common ancestry, by virtue of their shared chromosome number and size.

If the currently accepted systematic relationships among pleurocerid taxa accurately reflect their evolutionary history, one of the three karyotypes displayed in Figure 1 (*P. unciale, G. simplex,* or *J. hemphilli/G. livescens*) would seem to be ancestral. Assigning ancestral status to any of the other eight karyotypes would imply polyphyletic genera. White (1978) has noted that the "overriding majority" of cytogeneticists tend to assume that centric fusion is more common than fission, and hence that higher chromosome numbers are ancestral. But White offers evidence to the contrary from both the well-studied butterflies and the Australian morabine grasshoppers. Thus it would be premature to draw a "root" on Figure 2, or to speculate regarding the direction of karyotypic evolution implied, absent data on the other three genera in this family, *Leptoxis, Lithasia,* and *Io.* Such data will be forthcoming in the final paper of this series.

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