behaviour could be a result of water temperature and not individual size. The squid *Loligo opalescens* Berry, however, is able to compensate for the slowing effect of colder temperatures on neuronal and muscular aspects of submerged escape jetting so that its maximum water jet velocity and distance travelled are actually greater at colder temperatures. If *Ommastrephes bartramii* is similarly capable of temperature compensation, the suggestion by Murata that flight ability in this species is limited by size and not temperature is likely to be correct.

While there is some question as to the exact nature of airborne jet propulsion in squids, we believe use of the term ‘flight’ is not wholly inappropriate. Morphological traits such as broad fins and arm membranes, as well as airborne behaviours including jetting and changes in arm posture suggest that flight has evolved in squid, most likely as an anti-predator behaviour. Such traits also suggest that flight represents a more active and intentional escape behaviour than simply gliding after an incidental exit from the water during escape jetting. Whether considered flight or gliding, however, this behaviour appears to be more widespread than previously thought.

**ACKNOWLEDGEMENTS**

Many thanks to C. Albrecht, R. Anderson, P. Callomon, R. Cipriani, H. Saito and M. Vecchione for assistance in gathering information and publications relating to flying squid, to K. Yano for translating Azuma (1981), and to two anonymous reviewers for very helpful comments. This is contribution no. 7 of the Hofstra University Marine Laboratory.

**REFERENCES**


**A cytogenetic study of the periwinkle *Littorina keenae* Rosewater, 1978**

**Gastropoda: Littorinidae**

Angelo Libertini¹, Renata Trisolini¹ and Suzanne Edmands²

1CNR Istituto di Scienze Marine, Riva 7 Martiri 1364/A, I-30122 Venezia, Italy;
2Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0371, USA

Reid assigned 173 species to the gastropod family Littorinidae. The systematics of this family has recently been revised and new genera and subgenera have been proposed on the base of phylogenetic analysis of morphological and molecular characters. Cytogenetic studies may also contribute to phylogenetic analysis by providing additional characters for phylogenetic tree construction. Unfortunately, cytogenetic knowledge of littorinids is limited to a few species (Table 1). The chromosome number, and sometimes the karyotype morphology, has been investigated in nine species, and in two of them, *Melarhaphe neritoides* (Linnaeus, 1758) and *Littorina (Neritina) saxatilis* (Olivi, 1792), sex chromosomes have been described. The genome size (GS) has been determined only for six species. In the present study the chromosome number, karyotype morphology, GS and the percentage of adenine–thymine DNA in the genome (AT%) were investigated in *Littorina (Planilittorina) keenae* Rosewater, 1978. GS and AT% were also determined in *M. neritoides* for comparison.

About 100 specimens of *Littorina (Planilittorina) keenae* were collected on the rocks at high tide level in San Pedro and Malibu (southern California, USA) in August 2002. Twenty individuals of *Melarhaphe neritoides* were sampled along the stony shores in Venice (northeastern Italy) in August 2002. Taxonomic nomenclature for Littorinidae is according to Reid and Williams et al. Chromosome preparations of *L. keenae* were obtained by air-drying from the male gonad, after an 18-h treatment in 0.01% w/v colchicine/filtered sea water solution. GS and nuclear AT DNA content were evaluated through flow cytometric assay performed on cell suspensions of the two periwinkle species obtained from deep-frozen gill and mantle, by means of a BRITE-HE cytometer (Bio-Rad Laboratories) equipped with a xenon-mercury lamp. Peripheral blood erythrocytes from chicken (2C GS = 2.50 pg, 2C AT DNA = 1.39 pg) were added to the periwinkle cell suspensions as internal standard. The nuclei were stained with propidium iodide (40 specimens of *L. keenae* and 11 of *M. neritoides*) and Hoechst 33258 (34 specimens of *L. keenae* and 11 of *M. neritoides*) for GS and AT DNA evaluation, respectively. For each sample at least
Table 1. Cytogenetical parameters in the caenogastropod family Littorinidae.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex chromosomes</th>
<th>Origin</th>
<th>AT%</th>
<th>FN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cenchritis muricatus</td>
<td></td>
<td>Japan</td>
<td>16</td>
<td>0.82</td>
</tr>
<tr>
<td>Littoraria strigata</td>
<td></td>
<td>Japan</td>
<td>16</td>
<td>1.00</td>
</tr>
<tr>
<td>(brevicula)</td>
<td></td>
<td>Russia</td>
<td>9</td>
<td>1.18</td>
</tr>
<tr>
<td>Littoraria irrorata</td>
<td></td>
<td>Unknown</td>
<td>21</td>
<td>1.35</td>
</tr>
<tr>
<td>Littorina (Littorina littorea)</td>
<td></td>
<td>Italy</td>
<td>7</td>
<td>1.35</td>
</tr>
<tr>
<td>Littorina (Neritrema saxatilis)</td>
<td></td>
<td>Italy</td>
<td>7</td>
<td>1.35</td>
</tr>
<tr>
<td>Littorina (Neritrema saxatilis)</td>
<td></td>
<td>Italy</td>
<td>7</td>
<td>1.35</td>
</tr>
<tr>
<td>Melarhaphe neritoides</td>
<td></td>
<td>Italy</td>
<td>7</td>
<td>0.81</td>
</tr>
<tr>
<td>Echinolittorina hawaiiensis</td>
<td></td>
<td>Italy</td>
<td>7</td>
<td>0.81</td>
</tr>
</tbody>
</table>

The seven remaining chromosome pairs (Fig. 1C, pairs 11–17) were considered as mono-armed because their centromere was terminally located. Among the littorinids cytogenetically studied so far, L. keenae has the most common chromosome number of n = 17, found in six species out of nine, and the most asymmetrical karyotype with the lowest FN (Table 1).

Through flow cytometry, the mean C values of GS and AT DNA of L. keenae were determined as 0.90 ± 0.017 pg and 0.48±0.013, respectively; calculated AT% was 55.1%. In M. neritoides, GS was 1.47 ± 0.046 pg, AT DNA 0.83 ± 0.015 pg, and AT% 55.5%. In a previous paper, a GS of 1.38±0.052 pg was reported for M. neritoides. The small discrepancy between the reported GS values may be due to differences in the methods used, such as: different reference species (blue mussel vs chicken), different equipment (laser vs lamp-eytrometer) and different methods used to preserve the samples (70% ethanol vs deep freezing). Nevertheless, M. neritoides is still the species of Littorinidae with the highest GS (Table 1). Conversely, L. keenae showed a GS value that is intermediate in the GS range of the family (0.67–1.38 pg) (Table 1). Among the Littorinimorpha, the GS range of Littorinoida is similar to that of Rissoidea (0.68–1.25) and lower than that of Naticoidea (1.50–2.40). M. neritoides and L. keenae have similar AT% (55.3% vs 53.1%) and thus their genomes are slightly richer in AT than in GC base pairs (calculated GC percentages are therefore 44.5% and 46.9%, respectively). Unfortunately, it was impossible to find comparable data on DNA base composition for other caenogastropod molluscs. In a previous study of DNA contents in pulmonate gastropods, significantly higher GS and GC% (greater than 40%) were found in terrestrial species as compared with aquatic ones. The enrichment of GC% in the

2,500 cells were examined, and the DNA index (mean channel number of the G1/G0 peak of the periwinkle cells over the mean channel number of the G1/G0 peak of the chicken cells) was evaluated after elaboration of the fluorescence data by means of Modfit software (Verity Software House). The average DNA indices among the analysed samples, multiplied by half of the DNA content of the standard, gave the haploid values (C-values) assigned to the investigated species (data are reported as mean ± standard deviation).

In Table 1 all available data on chromosome number, karyotype morphology, sex chromosomes, genome size (C-value) and AT DNA% in the littorinid species cytogenetically investigated so far have been summarized. Since classification of chromosomes differs from author to author with regard to subtelocentrics, acrocentrics and telocentrics, karyotype formulas in Table 1 were formatted strictly according to Levan et al. Reclassifying reported results on chromosome arm measurements, and/or evaluating directly the centromeric index of each chromosome pair from karyogram figures by the practical method proposed by Naranjo et al. Sub-telocentric chromosomes were considered as bi-armed for the calculation of the fundamental number of chromosome arms (FN).

From 85 (out of 110) metaphase I plate counts the haploid number 17 was determined for L. keenae. Spermatoocyte bivalents (Fig. 1A) were rod-, lozenge-, cross- or dumb-bell-shaped, thus testifying the presence of chiasmata. No heteromorphic bivalent or monovalent was noticed in any spermatoocyte plate, and therefore there was no evidence of male heterogamety in L. keenae. In the members of the family, two different male heterogametic sex chromosome mechanisms have been found in M. neritoides and L. saxatilis. The haploid number 17 was confirmed by the observation of 34 chromosomes in four out of six spermatogonial metaphases (Fig. 1B). An attempt to construct the karyotype of L. keenae on the base of the chromosomes extracted from Figure 1B is shown in Figure 1C. Ten chromosome pairs (Fig. 1C, pairs 1–10) appeared as bi-armed, indicating the presence of a median, sub-median or sub-terminal centromere. The seven remaining chromosome pairs (Fig. 1C, pairs 11–17) were considered as mono-armed because their centromere was terminally located. Among the Littorinimorpha, L. keenae has the most common chromosome number of n = 17, found in six species out of nine, and the most asymmetrical karyotype with the lowest FN (Table 1).
genome was argued to confer a greater physical stability to DNA, secured by the triple H-bonds in a GC pair and, therefore, was considered as an adaptive trait for terrestrial life. Resistance to desiccation is known to be extremely high among littorinids, which on most shores can be found higher up the intertidal zone than other caenogastropods. Specific adaptation of shell morphology and physiology has been described for high-intertidal periwinkles. The relatively high percentage of GC DNA in the genome of *M. neritoides* and *L. keena* (44.5% and 46.9%, respectively) might be considered as a further adaptation to air exposure.

Data on karyotype morphology and DNA contents are still too scanty for clearly outlining the evolutionary trends of cyogenetic characters among littorinids, although some preliminary indications may be argued. The chromosome number n = 17 might be expected to be the ancestral value, being shown by most of the studied species coming from very far geographical areas (Table 1). GS is more variable than chromosome number and the two parameters do not seem to be correlated. *Littorina saxatilis, L. keena* and *M. neritoides* have the same chromosome number, but differ in karyotype formula and FN, thus indicating that speciation was accompanied by karyotype rearrangements mainly due to inversions and/or translocations. At present, it is not clear if the slight differences in karyotype formula (mainly not affecting the FN) and presence/absence of sex chromosomes among different geographical populations of *L. saxatilis* and *M. neritoides* may be due to effective local chromosomal rearrangements or to different interpretation of chromosome observations by the authors. The presence of two different mechanism of sex chromosome determination may indicate that sex chromosomes were a new acquisition (apomorphic) in littorinids, as well as in other gastropods, and appeared independently in *L. saxatilis* and *M. neritoides*.

Further data on cyogenetical parameters on Littorinidae are necessary to confirm or contradict present hypotheses about the pathways of karyological evolution in this taxon, and to contribute to phylogenetic analysis by providing additional characters for phylogenetic tree construction.

The authors wish to thank Dr David G. Reid (Natural History Museum, London) for the taxonominical identification of *L. keena* specimens.

**REFERENCES**


*Figure 1. Littorina (Planilittorina) keena. A. Spermatocyte metaphase I plate. B. Spermatogonial metaphase plate. C. Karyogram made up with the chromosomes from B. The chromosome pairs from 1 to 10 are b-arm ed, the pairs from 11 to 17 are mono-armed. Scale bar = 10 μm.*