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Mode of reproduction, ploidy and fluctuating asymmetry: comparison of co-existing sexual and asexual freshwater snails

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ABSTRACT

We compared fluctuating asymmetry of ecologically similar sexual and asexual freshwater snails (*Potamopyrgus antipodarum*). The asexual snails are triploid apomictic parthenogens, whereas the sexual snails are diploid. Although earlier studies have shown that the average heterozygosity does not differ between sexuals and asexuals, higher ploidy level and accumulation of mutations may affect the fluctuating asymmetry of asexuals. However, it is unclear how large this genetic effect might be relative to the environmental effects; empirical studies of the effects of genetic stress on fluctuating asymmetry suggest that weak effects are to be expected. Our aim in this study was to contrast the magnitude of variation in fluctuating asymmetry due to environment to that due to ploidy and mode of reproduction. For this purpose, we assessed fluctuating asymmetry in radula morphology of co-existing sexual and asexual females, knowing that the asexuals represented a group of different clonal genotypes. For reference, we also assessed among-population variation in fluctuating asymmetry and contrasted this to among-individual variation in fluctuating asymmetry. The radula is a bilaterally symmetric feeding structure that is composed of repeated rows of teeth. We found significant among-individual variation in fluctuating asymmetry, indicating sufficiently powerful statistical analysis, but we found no statistically significant differences in fluctuating asymmetry between sympatric asexual and sexual snails, or among different snail populations. Our results suggest that genetic effects of ploidy and asexuality on fluctuating asymmetry are too small to be detected against the environmental variation. These results suggest that the asexual and sexual snails respond to external stress factors similarly, at least with respect to the level of fluctuating asymmetry.

Keywords: asexual reproduction, fluctuating asymmetry, mutation load, *Potamopyrgus antipodarum*, radula.

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INTRODUCTION

It is generally assumed that fluctuating asymmetry reflects developmental stability that decreases with environmental or genetic stress (Mather, 1957; Palmer and Strobeck, 1986; Parsons, 1992). Several processes may affect the expression of genetic stress, and it is likely that environmental and genetic stress factors interact in natural populations. For example, mutation accumulation (Bourguet, 2000; Moller, 2000), inbreeding depression (Waldmann, 1999; Gomendio *et al.*, 2000), level of ploidy (Leary *et al.*, 1985; Mesaros *et al.*, 1994; Crespi and Vanderkist, 1997) and hybridization (Blows and Sokolowski, 1995; Wilsey *et al.*, 1998; Hochwender and Fritz, 1999) have all been suspected, or shown, to affect the level of fluctuating asymmetry. The results of empirical studies are somewhat inconsistent, but reviews have shown that weak effects of these genetic processes are to be expected (for a review of all fluctuating asymmetry reviews, see Palmer, 2000). There is general agreement that environmental stress increases fluctuating asymmetry, but only a few studies have reported a positive association between fluctuating asymmetry and a genetic stress factor (Britten, 1996; Sheridan and Pomiankowski, 1997; Palmer, 2000).

Our motivation for this study stems from a lack of empirical studies addressing the maintenance of sexual reproduction (Kondrashov, 1993). We wished to compare the fluctuating asymmetry of sympatric asexual and sexual snails to add to our understanding of the processes that maintain sexual morphs in our study system. Our study system, the New Zealand freshwater snail *Potamopyrgus antipodarum*, has been used to address the theories for maintenance of sex (Lively, 1996; Lively *et al.*, 1998; Jokela *et al.*, 1999). Previous studies have reported that the sympatric sexual and asexual snails are ecologically similar (Jokela *et al.*, 1997; Lively *et al.*, 1998), but not all aspects can be covered in single studies; therefore, we focus on fluctuating asymmetry here.

The aim of this study is to determine if the asexual triploid parthenogens differ from the diploid sexual snails in terms of level of fluctuating asymmetry. There are several reasons why this should be expected. First, asexual populations are expected to have a higher mutation load at mutation–selection balance than sympatric sexual populations, if sex is maintained as envisaged in the Mutational Deterministic Theory for sex (Kondrashov, 1988). Intuitively, one would expect mutation load to increase developmental instability and fluctuating asymmetry, but it is not clear if this actually happens (Monedero *et al.*, 1997). In our case, this is especially difficult to assess because we do not know if the asexual and sexual snails differ in their average mutation load. We only know that, under Mutational Deterministic Theory, asexuals should carry a higher mutation load. Furthermore, in a recent study, Bourguet (2000) reported that classes of *Drosophila* carrying different numbers of marker mutations differ by their fluctuating asymmetry, suggesting that mutations may directly affect the level of fluctuating asymmetry. Second, ploidy is expected to affect gene expression, which may affect developmental stability and fluctuating asymmetry. We know of five studies in which the magnitude of fluctuating asymmetry was compared based on ploidy. In two of these studies, a significant effect was found. Leary *et al.* (1985) reported that triploid rainbow trout have higher levels of fluctuating asymmetry than the diploids; Mesaros *et al.* (1994) reported that weevils with a higher ploidy level (triploid and tetraploid) have less fluctuating asymmetry than diploids. The remaining three studies compared males of haplo-diploid insects to diploid workers and found that the fluctuating asymmetry of some, but not all, traits differed in terms of ploidy (Crespi and Vanderkist, 1997), that the effect of ploidy was generally small (Smith *et al.*, 1997), or found no effect (Kudo and Mori, 2000).

In a recent study, Pertoldi *et al.* (2001) found that asexual stick insects (*Bacillus rossius*) expressed higher levels of fluctuating asymmetry than the ancestral sexual species. In these stick insects, the parthenogens are completely homozygous for all loci due to the form of parthenogenesis (Pertoldi *et al.*, 2001). Therefore, the authors suspected that the result was due to heterozygotes (sexuals) being better buffered against environmental variation, and called for studies of parthenogens where heterozygosity would be preserved. In *Potamopyrgus antipodarum*, sexuals and asexuals do not differ by their average heterozygosity because of the form of apomictic parthenogesis that involves an increase in ploidy (Dybdahl and Lively, 1995; Fox *et al.*, 1996).

The past decades have produced a multitude of methods to estimate fluctuating asymmetry. At present, fluctuating asymmetry studies are reviewed perhaps more critically than any other studies in the field of evolutionary biology (Palmer, 2000). This technical scrutiny has left us with reliably good agreement on how to estimate fluctuating asymmetry and on the potential problems associated with this (Palmer and Strobeck, 1986; Palmer, 1994, 1996). We used the radula morphology of *Potamopyrgus antipodarum* to estimate fluctuating asymmetry in sexual and asexual snails. The radula is a bilaterally symmetric feeding apparatus of gastropods that is composed of repeated rows of teeth (Fig. 1). The structure is ideal for studies of fluctuating asymmetry because: (1) several traits can be measured simultaneously; (2) the structure is repeated; and (3) new rows of teeth grow as the teeth used for feeding wear out. Additionally, (4) the repeated structure of the radula allows reliable estimation of within-individual variance in fluctuating asymmetry.

We report considerable variation in the fluctuating asymmetry of the radula among individual snails. We compared fluctuating asymmetry in samples of snails using a composite asymmetry score that was based on seven morphological traits. We did not find significant differences in fluctuating asymmetry between asexual and sexual individuals, nor did we find significant differences among genetically and ecologically different snail populations. These negative results were not due to a lack of statistical power; on the contrary, the level of fluctuating asymmetry observed among individuals was considerable. Hence, these results suggest that triploidy and the asexual mode of reproduction has not lead to genetic effects that would have altered the level of fluctuating asymmetry in these snails.

MATERIALS AND METHODS

Study organism

The freshwater snail, *Potamopyrgus antipodarum*, is a unique organism in that abundant mixed populations of obligatorily sexual and obligatorily asexual individuals are commonly found in diverse freshwater habitats of New Zealand (Lively, 1987; Dybdahl and Lively, 1995). Because reproductive morphs compete within the mixed populations, these prosobranch snails have been used as a model system in empirical studies of the evolutionary maintenance of sex (for a recent review, see Lively, 1996). Genetic data suggest that asexual snails originate from differentiated sexual populations (Dybdahl and Lively, 1995), and often the clonal diversity in mixed populations is high (Dybdahl and Lively, 1995; Fox *et al.*, 1996). However, the age of the clones is not known. The snail populations vary from completely clonal to completely sexual, and the frequency of sexual individuals has been shown to correlate positively with the prevalence of parasite infections (Lively, 1987, 1992; Jokela and Lively, 1995a).

The radula

The radula is an elongated ribbon-shaped feeding structure of gastropods, composed of hundreds of repeated rows of teeth (Fig. 1). The teeth have indentations and special morphological characteristics widely used in gastropod taxonomy. Teeth morphology may vary considerably even within a species (for examples, see Hickman, 1980; Houbrick, 1991; Hershler, 1994). The radula is bilaterally symmetrical: each morphological trait can be measured from the left and right side of the row of teeth, and all structures are repeated in each row of teeth. The teeth are used to scrape food from surfaces and wear out from the front end of the radula (Thomas *et al.*, 1985). Behind the actively scraping teeth, new rows of teeth develop continuously (Fujioka, 1985). The whole radula may be regenerated several times in a year (Fujioka, 1985).

The traits that we measured from the radula are illustrated in Fig. 1. We measured 11 traits from each of four teeth per individual snail. The teeth were chosen from the middle section of the radula, because in this section the teeth are fully developed, but not yet used for feeding. The radula were extracted from snails by immersing the snails in KOH for a maximum of 20 h. The radula is chitinized and does not dissolve readily in KOH. However, during immersion, the snails were inspected repeatedly to prevent damage to the radula. After extraction, the radula were washed and preserved in alcohol and then prepared for scanning electron microscopy (SEM) using standard procedures. When a suitable view

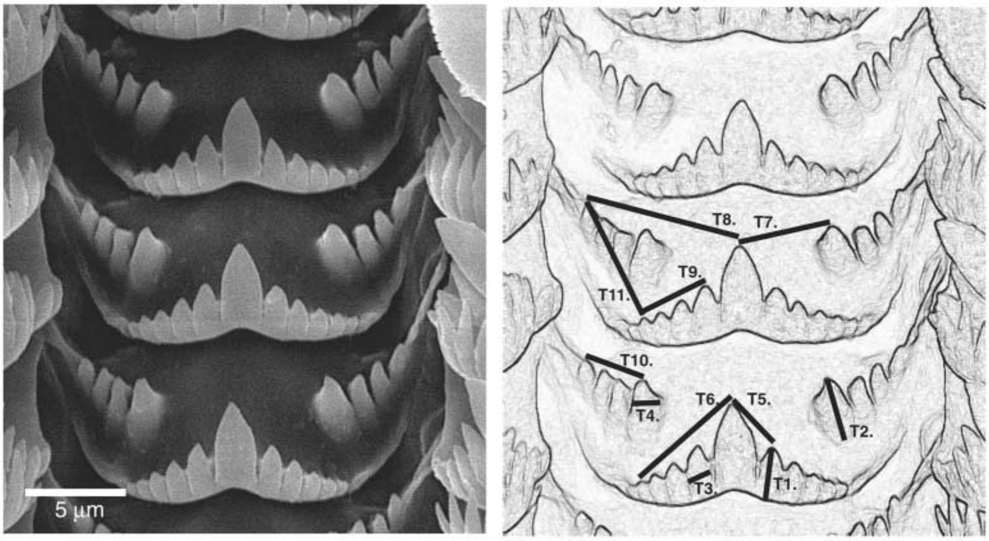

Fig. 1. SEM image of the radula of *Potamopyrgus antipodarum* (scale bar = 5 μm). Left panel shows three central teeth. Right panel illustrates the 11 traits that were measured from each of the four teeth per individual.

(showing several rows of teeth) directly from the top of the radula was available (Fig. 1), a digitized picture of the radula was transferred on to a computer disk. When choosing the view, special attention was paid to the position of the radula on the mounting stub. Failure to shoot the images from directly above the sample would increase the estimates of antisymmetry (see below), and may decrease the power to estimate fluctuating asymmetry. The teeth were later measured from these digitized pictures using NIH image analysis software (US National Institutes of Health, available at http://rsb.info.nih.gov/nih-image/). The same person took all the measurements.

Estimation of fluctuating asymmetry

We assessed measurement error in the morphological traits by repeating the measurements for eight randomly chosen snails several weeks after the original data had been collected. We used a mixed-model analysis of variance to estimate the magnitude of measurement error in each fluctuating asymmetry score (Palmer and Strobeck, 1986; Palmer, 1994). A mixed-model analysis of variance was conducted as recommended by Palmer and Strobeck (1986, table 3a). In this analysis, replicate measures from a single individual (the four teeth measured twice) were used to estimate size variation among individuals. The side of the measurement (left or right) was included as a factor to assess the magnitude of directional asymmetry. ‘Directional asymmetry’ means that organisms are not symmetric for the measured traits; that is, in a sample of organisms, one of the two sides (e.g. the left side) is consistently larger than the other side (Palmer and Strobeck, 1986). A Side × Individual interaction reflects type 1 antisymmetry, while variation among the teeth of the same individual (Tooth, nested under the Side × Individual interaction) includes the fluctuating asymmetry and type 2 antisymmetry (Palmer and Strobeck, 1986). These types of antisymmetry refer to the tendency for the two sides of organisms in a sample to be of different sizes, but that the larger of the sides (left or right) is determined at random for each individual. When antisymmetry is present, the distribution of the difference in size between the sides does not follow a normal distribution, but rather shows a broad-peaked distribution, or even bimodality (Palmer and Strobeck, 1986). Measurement error may be estimated from this analysis as the within-teeth variance that is used as the error term in the test assessing the significance of fluctuating asymmetry (Palmer and Strobeck, 1986).

We conducted an additional analysis to assess the reliability of the unsigned fluctuating asymmetry scores that were used for comparison of the samples. Because we measured four teeth per individual, it was possible to calculate the intraclass correlation coefficients (Fisher, 1918) for each of the 11 fluctuating asymmetry scores. The intraclass correlation coefficient is used as an index of the repeatability of the measurements taken from one individual. It is calculated as the proportion of between-individual variation of the total phenotypic variation (Lynch and Walsh, 1998):

$$r = \frac{\text{var(between)}}{\text{var(total)}}$$

We estimated the required variance components using a one-way analysis of variance where individuals were treated as a fixed factor. Following Lynch and Walsh (1998), the non-biased variance components were then calculated as:

$$\text{var(between)} = \frac{\text{MS}_{\text{between}} - \text{MS}_{\text{within}}}{n}$$

$$\text{var(within)} = \text{MS}_{\text{within}}$$

$$\text{var(total)} = \text{var(between)} + \text{var(within)}$$

where n = the number of measurements per individual (the four teeth).

We then plotted the estimated measurement error for each trait against the repeatability of the trait. High repeatability indicates that the individual fluctuating asymmetry estimates are measured with low measurement error (Fig. 2). Independent data sets were used to calculate these estimates; measurement error was estimated using the eight snails measured twice, as described above, and repeatability was calculated using the pooled data collected for the comparison of sexual and asexual individuals ($n = 38$ individuals; see below).

The purpose of these analyses was to reveal which of the measured traits are the most reliable estimates of fluctuating asymmetry. Based on the analyses, we chose a subset of traits for the comparison of fluctuating asymmetry scores calculated for asexual and sexual snails, and for the comparison of samples from different populations (see below). We chose the seven traits that were measured with less than 40% measurement error (Fig. 2); the 40% threshold was an arbitrary choice. Additionally, we calculated a composite fluctuating

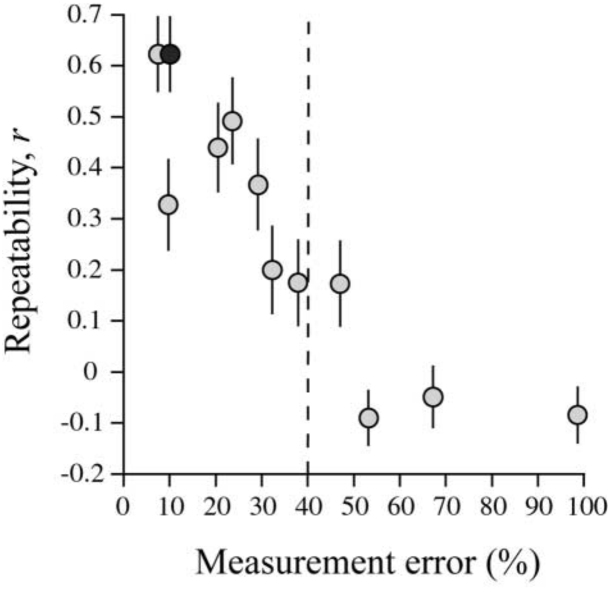

Fig. 2. Relationship between measurement error and repeatability of the 11 morphological traits measured. The black dot indicates the fluctuating asymmetry index (Table 3) that was calculated based on the traits that had <40% measurement error (indicated by a dashed line). Standard error (SE) for the repeatability coefficients was calculated as $\text{SE}(r) \cong \sqrt{\frac{2(1-r)^2[1+n-1\,r]^2}{Nn(n-1)}}$, where N = number of individuals and n = number of teeth measured per individual (Lynch and Walsh, 1998).

asymmetry index based on the trait values of each individual. For all these comparisons we used the absolute value of the difference between the right and left side of the radula as a measure of fluctuating asymmetry.

Study populations

The sample of asexual and sexual snails was collected in February 1999 from the shallow water habitat of the 'Camp' site at Lake Alexandrina (South Island, New Zealand) (for a map of sites, see Jokela and Lively, 1995b). Based on previous studies, we knew that this sample would consist of a mixture of sexual and asexual snails (Fox *et al.*, 1996). The snails were kept alive in the laboratory at Zürich, Switzerland, until July when they were prepared for radula measurements. Asexual snails differ from sexual snails by being triploid (Wallace, 1992; Dybdahl and Lively, 1995). The ploidy level was identified with standard cellulose acetate electrophoresis using two loci: 6-phosphogluconate dehydrogenase (6PGD) (EC 1.1.1.44) and phosphoglucomutase (PGM) (EC 2.7.5.1).

We compared the fluctuating asymmetry of eight *P. antipodarum* populations. Samples of these populations were collected in early 1998 and 1999 and kept in the laboratory until the preparation of radula (July 1999). The available background information of the samples and sample sizes used in the different analyses are presented in Table 1. We used a nested analysis of variance to test for the differences in fluctuating asymmetry between asexual and clonal snails and among the eight populations. In these analyses, individual snails from each population were nested within the grouping variable (sexual/asexual or population). The error variance was calculated as the within-individual variance based on the four teeth measured from each individual.

RESULTS

Fluctuating asymmetry and measurement error

Mixed-model analyses of variance (Table 2) indicated that the magnitude of non-directional asymmetry (including fluctuating asymmetry and one of the two forms of anti-symmery) was statistically significant in all traits measured except for trait 4, the basal width of the first hind-cusp (Fig. 1, Table 3). The analysis did not reveal significant directional asymmetry (F-test, $P > 0.05$ for all 11 traits). An additional analysis of signed asymmetry scores (left − right value) revealed no significant deviation from normality (Kolmogorov-Smirnov test, $P > 0.05$, followed by graphical p–p plot analysis), suggesting that antisymmetry was not significant. This result was further corroborated by plotting the unsigned asymmetry score [ABS(left − right)] against the mean trait size [(left + right)/2], as suggested by Rowe *et al.* (1997). None of the plots suggested significant antisymmetry, or an association between fluctuating asymmetry score values and size of the trait (data not shown).

The measurement error was higher than 40% for four of the 11 traits (Table 3). As expected, traits that were estimated with low measurement error showed high repeatability coefficients (Fig. 2). We excluded the four traits with the highest measurement error from further analyses, and combined the remaining seven traits into one measure of asymmetry by summing the absolute differences of the left and right side of each trait. We used this index in the following comparisons of the samples to illustrate the patterns. The results remained qualitatively similar when analyses were conducted with the original traits.

Table 1. Samples used for comparison of fluctuating asymmetry of sexual and asexual *P. antipodarum*, and among the eight study populations

Sample	Origin	Habitat	*n*	Males	Collected
'Asexual'	Lake Alexandrina	Shallow (0.5 m)	19	—	February 1999
'Sexual'	Lake Alexandrina	Shallow (0.5 m)	19	—	February 1999
Population 1	Lake Alexandrina	Deep habitat (5 m)	6	20%	February 1999
Population 2	Lake Alexandrina	*Isoetes* macrophytes (2 m)	6	50%	February 1999
Population 3	Lake Alexandrina	Shallow (0.5 m)	6	50–80%	February 1999
Population 4	Canterbury	Stream	6	0%	February 1998
Population 5	Grasmere	Stream	6	—	February 1999
Population 6	Oaro	Stream	6	0%	February 1998
Population 7	Lake Mapourika	Shallow (0.5 m)	6	30%	February 1999
Population 8	Lake Poerua	Shallow (0.5 m)	6	0%	February 1999

Note: *n* indicates sample size. 'Males' indicates proportion of males in the population. Proportion of males increases as the proportion of sexual snails increases (Fox *et al.*, 1996).

Table 2. Results of a mixed-model analysis of variance used to estimate the magnitude of fluctuating asymmetry and measurement error in morphological traits of *P. antipodarum*

Effect	d.f.	MS	*F*	*P*
Side (S)	1	4 577	0.02^a	0.897
Individual (*T*)	7	1 994 444	108.29^b	< 0.001
$S \times I^a$	7	252 496	13.71^b	< 0.001
$\text{Tooth}(S \times I)^b$	48	18 418	18.79^c	< 0.001
Error^c	64	980		

Note: The table illustrates the results for the composite fluctuating asymmetry variable calculated based on seven fluctuating asymmetry traits (see text and Table 3). A similar analysis was conducted for all 11 traits; the results are summarized in Table 3. Superscripts indicate the error term for *F*-tests. 'Side' was treated as a fixed factor and 'Individual' and 'Tooth' as random factors. Effect 'Tooth(S × I)' includes fluctuating asymmetry and type 2 antisymmetry.

Fluctuating asymmetry of sexual and clonal snails, and variation in fluctuating asymmetry among snail populations

Comparison of asexual and sexual snails revealed significant variation in the fluctuating asymmetry scores among individuals, but no significant difference in the average fluctuating asymmetry between sexual and asexual snails (Table 4, Fig. 3). The results were similar when samples from eight different populations were compared (Table 5). Differences in fluctuating asymmetry among individuals within populations were larger than the average differences between populations (Fig. 4, Table 5). In other words, the nested analysis suggests that the among-individual variation in fluctuating asymmetry clearly exceeds the variation that is observed among the populations, or between sexual and asexual snails.

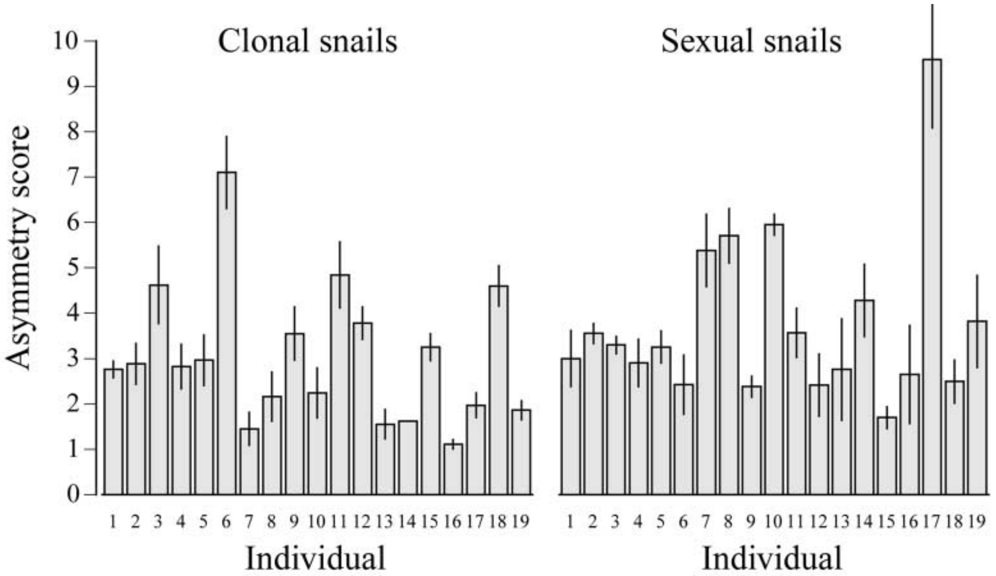

Fig. 3. Values of the fluctuating asymmetry index (±1 SE) calculated for clonal and sexual *P. antipodarum*. The fluctuating asymmetry index is a composite variable of seven morphological traits of the radula.

Table 3. Summary of the results of mixed-model analyses of variance for each morphological trait illustrated in Fig. 1

	MS_t	MS_e	*P*	ME%	Fluctuating asymmetry/ Total (%)
Trait 1	634	195	< 0.001	47.0	5.83
Trait 2	2 082	753	< 0.001	53.2	15.13
Trait 3	164	83	0.007	67.2	9.14
Trait 4	540	525	0.454	98.6	0.41
Trait 5	329	77	< 0.001	37.9	7.04
Trait 6	1 385	158	< 0.001	20.5	8.04
Trait 7	1 751	89	< 0.001	9.7	11.83
Trait 8	2 355	92	< 0.001	7.5	15.91
Trait 9	1 412	189	< 0.001	23.6	20.70
Trait 10	800	137	< 0.001	29.2	19.64
Trait 11	1 343	259	< 0.001	32.3	8.73
Fluctuating asymmetry index	18 418	980	< 0.001	10.1	6.64

Note: MS_t indicates the mean square of the nested effect 'tooth' that includes the variance due to fluctuating asymmetry. MS_e is the error mean square that indicates the magnitude of the variance component due to measurement error. Column *P* depicts the significance of fluctuating asymmetry (*F*-test, MS_t/MS_e). Measurement error (column 'ME%') is calculated as the proportion of variance due to measurement error to the total variance, i.e. $ME = 100 \times MS_e/[MS_e + (MS_t - MS_e)/n]$(Merilä and Björklund, 1995), where *n* is the number of measurements ($n = 2$). The last column gives the proportion of the total variance explained by fluctuating asymmetry $[(MS_t - MS_e)/2]$. 'Fluctuating asymmetry index' is the composite fluctuating asymmetry score that was calculated as the sum of traits 5–11.

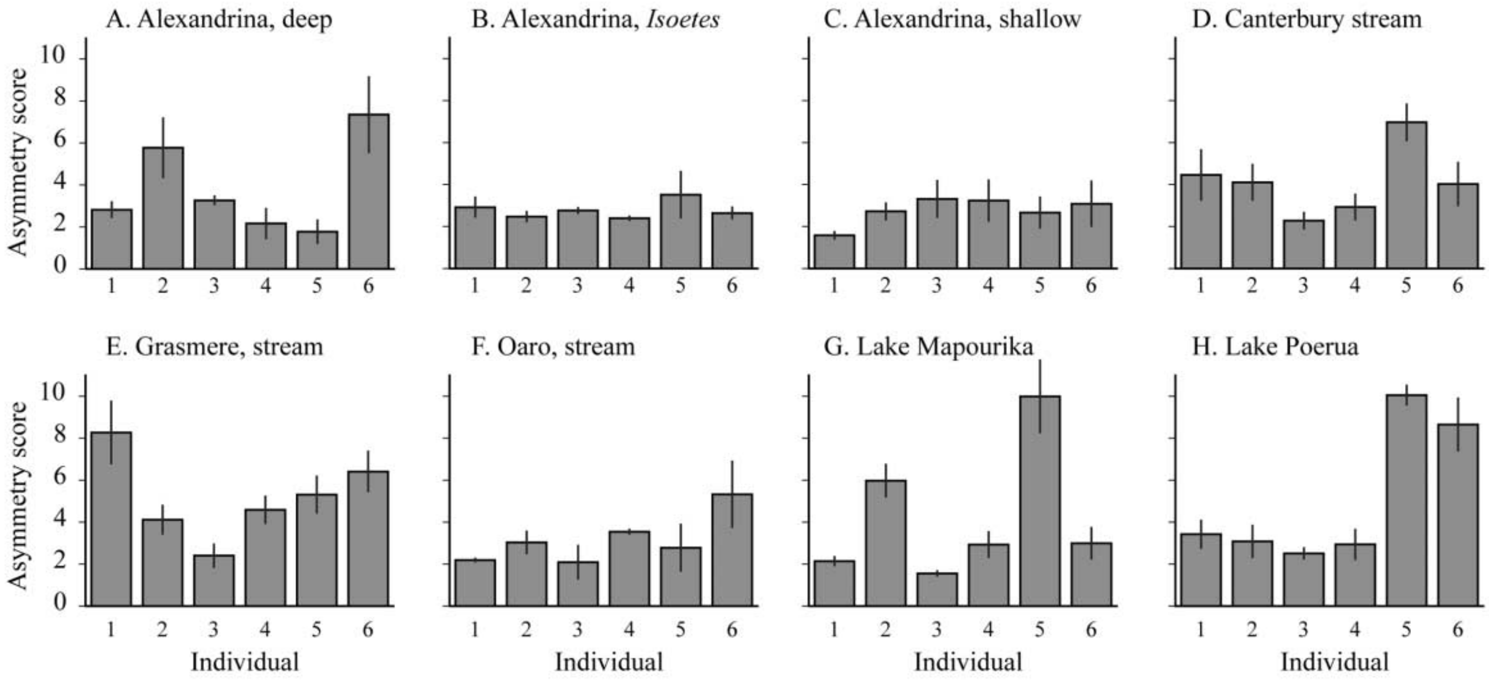

Fig. 4. Values of the fluctuating asymmetry index (±1 SE) calculated for samples of *P. antipodarum* from different populations. The fluctuating asymmetry index is a composite variable of seven morphological traits of the radula.

Table 4. Results of nested analysis of variance in which variation in the composite measure of fluctuating asymmetry ('fluctuating asymmetry index' in Table 3, see text for details) among individuals (IND) within reproductive modes (REPMO) was contrasted to the overall variation between sexual and asexual snails

Effect	MS	d.f.	*F*	*P*
REPMO	5.39	1	0.60	0.445
IND(REPMO)	9.04	36	7.50	< 0.001
Error	1.20	109		

Note: Significance of the variance due to reproductive mode is tested against the mean square of the nested effect.

Table 5. Results of nested analysis of variance in which variation in the composite measure of fluctuating asymmetry ('fluctuating asymmetry index' in Table 3, see text for details) among individuals (IND) within populations (POP) was contrasted to the overall variation between populations

Effect	MS	d.f.	*F*	*P*
POP	21.08	1	1.24	0.304
IND(POP)	17.00	40	5.98	< 0.001
Error	2.85	109		

Note: Significance of the variance due to population is tested against the mean square of the nested effect.

DISCUSSION

Our analysis revealed considerable variation in the level of fluctuating asymmetry among individual snails, but no statistically significant differences between sexual and asexual snails, or among different snail populations. This suggests that the genetic effects of triploidy and asexual mode of reproduction on fluctuating asymmetry are too small to be detected against the environmental variation. Furthermore, the among-population differences in fluctuating asymmetry were smaller than the among-individual effects, suggesting that a large-scale external environment is less important than processes at the individual level. Here, environmental variation includes variation induced both in the laboratory (some snail populations were maintained in laboratory cultures for a year before the measurement of fluctuating asymmetry) and in the wild (snails were wild-caught in the comparison of ploidy).

Increased ploidy may mask recessive mutations and increase developmental stability, as observed by Mesaros *et al.* (1994). Similarly, the increased heterozygosity that is involved with hybridization events has been reported to improve developmental stability of hybrids in some cases (Alibert *et al.*, 1994). An interesting example comes from the study of Vrijenhoek and Lerman (1982), who compared heterozygosity and fluctuating asymmetry of co-existing sexually and clonally reproducing fish. They found that inbreeding due to population crashes, migration barriers and environmental fluctuation had led to increased fluctuating asymmetry of the sexual populations with increased homozygosity, but that the co-existing clonal fish did not show a similar pattern because their heterozygosity was conserved after the initial hybridization event that gave rise to each clonal strain. Furthermore, clonal fish did not differ in their fluctuating asymmetry from the ancestral sexual fish, even though they expressed clearly higher average heterozygosity. In another case study, homozygosity was suspected to be the reason for increased fluctuating asymmetry in parthenogenetic stick insects when compared to more heterozygous sexual forms (Pertoldi *et al.*, 2001).

In *P. antipodarum*, the heterozygosity of parthenogens does not differ from that of sexuals, and the parthenogens are derived from local sexual populations (no interpopulation or interspecific hybridization) (Dybdahl and Lively, 1995; Fox *et al.*, 1996). Therefore, parthenogens and sexuals express their genes in roughly similar genetic backgrounds, the exception being that parthenogens are of higher ploidy. If the asexuals in this system are evolutionarily 'young' (as local origin might suggest), there might not have been enough time for mutation accumulation to differentiate the clones from the sexuals. If this is the case, our results suggest that the number of copies of genes (ploidy difference between asexuals and sexuals) does not affect fluctuating asymmetry. Although most asexuals are considered to have a recent origin (Judson and Normark, 1996), it is difficult to assess how representative our case would be when compared to other systems of co-existing sexual and asexual lineages.

Although it may be possible that the asexuals in this system are evolutionarily young (unfortunately at present we do not have good estimates of their age), the ageing of clones will result in mutations. Given enough time, clonal populations will be maintained at mutation–selection balance. Assuming mutation–selection balance is critical for the Mutational Deterministic hypothesis for the maintenance of sex (Kondrashov, 1988). It is also important to consider how ploidy may affect fluctuating asymmetry at mutation–selection balance. If masking of mutations takes place, it simply means that reaching the mutation–selection balance takes longer for triploids than for diploids. After mutation–selection balance has been reached, parthenogens should be maintained at higher mutation load, given synergistic epistasis between deleterious mutations (Kondrashov, 1988), and any differences in the masking of mutations by ploidy would not matter for the expression of developmental instability. It is not possible to conclude from our results what is the mutation load of the parthenogens and sexuals. What our results show is that whatever the magnitude of genetic effects in fluctuating asymmetry are in this system: (1) the effects are smaller than the environmentally induced variation in fluctuating asymmetry and (2) parthenogens and sexuals express similar levels of fluctuating asymmetry.

In an earlier study, Lively *et al.* (1998) found that parthenogens and sexuals responded similarly to environmental stress, suggesting that mutation load might not be that different. In another study, Jokela *et al.* (1997) found that parthenogens and sexuals were identical with respect to life-history traits and that sexuals pay the cost of sex in this system. Our results are in line with those of these earlier studies, as parthenogens and sexuals appear similar with respect to the expression of developmental instability.

To our knowledge, the snail radula has rarely been used to estimate fluctuating asymmetry, although phenotypic variation in radula morphology has been suggested to reflect fluctuating asymmetry (Taylor and Lewis, 1995). Our results suggest that it is well-suited for studies of fluctuating asymmetry. One advantage of the radula is its repeated structure. Often, fluctuating asymmetry is measured in traits that are present only as single copies per individual; therefore, the estimates of within-individual variance in fluctuating asymmetry are problematic (Van Dongen, 1998; Whitlock, 1998). Our analysis indicates that replication of traits within individuals allows a powerful analysis of the individual-level variation in fluctuating asymmetry, which was further aided by the high number of traits available. Digitized SEM images also allowed high-precision measurements and relatively reliable replication. Because all gastropods have a radula, fluctuating asymmetry studies in these organisms are possible using techniques similar to those in the present study. We suspect that fluctuating asymmetry studies in other gastropods – for example, in hermaphroditic snails that have a variable mating system – would add considerably to empirical tests of various fluctuating asymmetry hypotheses. Furthermore, comparison of habitually selfing species to species that outcross might allow an interesting comparison.

In summary, our results suggest that the genetic differences between the parthenogens and sexuals are not large enough to reflect on the fluctuating asymmetry in this system. The simplest explanation for this would be that fluctuating asymmetry does not have a genetic component, but is solely determined by environmental factors. If we accept that fluctuating asymmetry reflects the level of genetic stress, our results suggest that genetic stress is not different between parthenogenetic and sexual snails. These results are important for understanding how sexual morphs are maintained in this system, as they support previous results emphasizing the ecological similarity of co-existing sexual and parthenogenetic morphs (Jokela *et al.*, 1997). Previous results with this system have been in agreement with many of the assumptions of the Red Queen hypothesis, emphasizing the role of antagonistic frequency-dependent selection by parasites for the maintenance of sex (Lively, 1987, 1992; Jokela and Lively, 1995a; Dybdahl and Lively, 1998; Lively and Dybdahl, 2000). Whether or not the Red Queen is the explanation for sex in this system will have to be resolved by detailed studies of the dynamics of individual clones and their vulnerability to infection in natural populations. Our results, together with those of previous studies, suggest that, as a group, the triploid parthenogens do not differ from diploid sexuals in any other respect than in the production of males. This implies that the higher ploidy level *per se* does not counterbalance the cost of sex in this system.

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