

Intraclonal genetic variation: ecological and evolutionary aspects.

Edited by H. D. Loxdale FLS, FRES and G. Lushai FRES

Genetic variation in sexual and clonal lineages of a freshwater snail

JUKKA JOKELA^{1,*}, CURTIS M. LIVELY², MARK F. DYBDAHL³ and JENNIFER A. FOX⁴

¹*Department of Biology, University of Oulu, PO Box 3000, FIN-90014 University of Oulu, Finland*

²*Department of Biology, Indiana University, Bloomington, IN 47405–3700, USA*

³*School of Biological Sciences, PO Box 644236, Washington State University, Pullman, WA 99164–4236, USA*

⁴*Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14850, USA*

Sexual reproduction within natural populations of most plants and animals continues to remain an enigma in evolutionary biology. That the enigma persists is not for lack of testable hypotheses but rather because of the lack of suitable study systems in which sexual and asexual females coexist. Here we review our studies on one such organism, the freshwater snail *Potamopyrgus antipodarum* (Gray). We also present new data that bear on hypotheses for the maintenance of sex and its relationship to clonal diversity. We have found that sexual populations of the snail are composed of diploid females and males, while clonal populations are composed of a high diversity of triploid apomictic females. Sexual and asexual individuals coexist in stable frequencies in many 'mixed' populations; genetic data indicate that clones from these mixed populations originated from the local population of sexual individuals without interspecific hybridization. Field data show that clonal and sexual snails have completely overlapping life histories, but individual clonal genotypes are less variable than individuals from the sympatric sexual population. Field data also show segregation of clones among depth-specific habitat zones within a lake, but clonal diversity remains high even within habitats. A new laboratory experiment revealed extensive clonal variation in reproductive rate, a result which suggests that clonal diversity would be low in nature without some form of frequency-dependent selection. New results from a long-term field study of a natural, asexual population reveal that clonal diversity remained nearly constant over a 10-year period. Nonetheless, clonal turnover occurs, and it occurs in a manner that is consistent with parasite-mediated, frequency-dependent selection. Reciprocal cross-infection experiments have further shown that parasites are more infective to sympatric host snails than to allopatric snails, and that they are also more infective to common clones than rare clones within asexual host populations. Hence we suggest that sexual reproduction in these snails may be maintained, at least in part, by locally adapted parasites. Parasite-mediated selection possibly also contributes to the maintenance of local clonal diversity within habitats, while clonal selection may be responsible for the distribution of clones among habitats. © 2003 The Linnean Society of London. *Biological Journal of the Linnean Society* 2003, **79**, 165–181.

ADDITIONAL KEYWORDS: clonal diversity – coevolution – frozen-niche variation – maintenance of sex – parthenogenesis – *Potamopyrgus antipodarum* – Red Queen hypothesis – trematodes.

INTRODUCTION

Clonal reproduction has clear advantages over sexual reproduction. The growth rate of clonal lineages should exceed that of sexual lineages because all individuals are able to reproduce, unlike sexual lineages which contain males ('cost of males', Maynard Smith, 1978). Clones should also have a colonization advan-

tage (Baker, 1955), because they can establish populations from single individuals, unlike sexuals where the group of colonizers has to include both males and females. The reproductive advantage of clones over sexuals is therefore considerable, and should lead to rapid exclusion of sexuals in natural populations. For example, a single clonal individual introduced into a population of 10^6 sexual individuals is able to replace the sexual population in just 50 generations (Lively, 1996). Although the theory predicts that clones are

*Corresponding author. E-mail: jukka.jokela@oulu.fi

able to eliminate coexisting sexual populations, sexual species dominate the world, and clonally reproducing organisms are considered special cases (Bell, 1982).

A large body of theory seeks to explain the failure of clones, given their apparent reproductive advantage (Williams, 1975; Maynard Smith, 1978; Bell, 1982; Hamilton, Axelrod & Tanese, 1990; Kondrashov, 1993). Our primary purpose in this paper is to explore the processes that promote and maintain sexual reproduction where a diverse mixture of clones coexists with sexual individuals. Such populations are common in the New Zealand freshwater snail, *Potamopyrgus antipodarum* (Gray) (Mollusca: Gastropoda: Hydrobiidae). Our secondary purpose is to understand the mechanisms that lead to the high levels of clonal diversity observed within and among populations of this snail.

MAINTENANCE OF SEX

ECOLOGICAL MODELS

The ecological theories for the maintenance of sex fall naturally into three categories that depend on the type of selection. In Williams' (1975) 'lottery model', selection is independent of both frequency and density ('hard' selection). The basic idea here is that outcrossed sexual reproduction is favoured over asexual reproduction as a mechanism to reduce the among-year variance in offspring production. As such, sexual reproduction would increase the geometric mean fitness, and can be seen as a 'bet-hedging' strategy (Seger & Brockmann, 1987). Thus, under the lottery model sex would be favoured in temporally variable environments, especially if the environment occasionally changes dramatically, as such changes would eliminate narrowly adapted clones. However, as argued below, such selection might also favour clones with general-purpose genotypes or a high degree of phenotypic plasticity.

The 'tangled bank model', in contrast, depends on selection that is both frequency- and density-dependent ('soft' selection). In fact, the frequency dependence in the model is driven by competitive interactions at high density. Originally formulated by Ghiselin (1974) and later expanded by Bell (1982), this model predicts that sex would be favoured in multiple-resource environments where competition for resources is intense. Such competition would favour rare phenotypes that specialize on the under-utilized resources, and can select for a stable genetic polymorphism, as originally shown by Levene (1953). The basic idea is that selection that favours the production of polymorphic offspring might also favour sexual over asexual reproduction as a mechanism for the production of variable offspring. However, the conditions for a stable genetic polymorphism can be quite narrow

(Maynard Smith & Hoekstra, 1980; Lively, 1986; 1999). The tangled bank model is conceptually similar to the 'frozen-niche variation hypothesis' of Vrijenhoek (1979, 1984), although the latter is more focused on the maintenance of clonal diversity than sex *per se*. Both hypotheses rely on narrowly specialized clonal genotypes, and both have a Levene-type multiple niche polymorphism at their core.

Lastly, the 'antagonistic coevolution' or 'Red Queen' hypothesis relies on selection that is strongly frequency dependent, and lagged in time. The basic idea of this hypothesis is that, as any host genotype becomes common, there is increasing selection favouring parasite genotype(s) that can infect it (Haldane, 1949). If the parasites (or biological antagonists, in general) are sufficiently virulent, the common host genotype is driven down in frequency, leading to the possibility of oscillatory dynamics (Jayakar, 1970; Clarke, 1976). The model is easily extended to the evolutionary maintenance of sex, since genetically specialized parasites should select against clonal mutants in a sexual population, once the clones become common (Jaenike, 1978; Hamilton, 1980, 1982; Hamilton *et al.*, 1990; Howard & Lively, 1994, 1998). A recent study also suggests that selection imposed by the host can in turn favour sexual reproduction in the parasite (Howard & Lively, 2002).

Parasite-mediated selection might also lead to the coexistence of different clonal genotypes, even if the competing clones have different fecundities, and they do not specialize on different resources. Theoretical studies have also shown that, with repeated mutation to asexual reproduction within the local sexual population, host-parasite coevolution can lead to the accumulation of clones with different resistance genotypes; such accumulation of clones can in turn lead to the eventual elimination of the ancestral sexual population (Lively & Howard, 1994). Hence, parasites might contribute to the evolutionary maintenance of sexual reproduction in their hosts (and vice versa), but they might not be sufficient. However, recent models have shown that antagonistic coevolution in combination with mutation accumulation in clonal lineages can act to eliminate both host and parasite clones in the short term (Howard & Lively, 1994, 1998, 2002).

MUTATION-ACCUMULATION MODELS

The mutation accumulation models can be roughly divided into two main types, depending on whether or not they rely on genetic drift to eliminate the clonal genotypes with the fewest mutations. In the original model, which came to be known as Muller's ratchet (Felsenstein, 1974), mutations accumulate in a clonal lineage as it spreads in the sexual population (Haigh, 1978; Bell, 1982, 1988a). This process leads to a dis-

tribution of mutations that is approximately normally distributed. As such, only one class contains the fewest number of mutations, and in small populations this class can be quite small and subject to genetic drift from two different sources (Howard & Lively, 1998). Firstly, each offspring produced by the members of this class might contain one or more additional mutations. Secondly, individuals in this 'least-loaded' class might fail to reproduce. Most likely, the two sources of drift would work together to eliminate the least-loaded class. Such elimination, however, only produces a new least-loaded class with slightly more mutations. The end result is that the clonal lineage accumulates more and more mutations, until the mutational load is such that individuals are barely able to replace themselves. At this point the population will periodically dip below carrying capacity, which further fuels Muller's 'ratchet-like process'. Lynch, Conery & Burger (1995a,b) refer to this final phase as the mutational meltdown, since the clones rapidly go to extinction.

There is now abundant evidence to suggest that Muller's ratchet does operate (Leslie & Vrijenhoek, 1980; Bell, 1988b; Chao, 1990; Rice, 1994; Moran, 1996), but at present it seems that the process does not operate fast enough (except in very small populations) to be a sufficient explanation for the evolutionary persistence of sex.

In the second mutation accumulation model, genetic drift may be important (although not required) in moving the clonal population to mutation-selection balance. However, drift becomes less important once this state is reached. The reason stems from the type of fitness function assumed in this newer model, which is called the mutational deterministic (MD) model (Kondrashov, 1988). In the MD model, the mutations are assumed to act in a synergistic way (rather than in an independent way as in Muller's ratchet) such that, at some point, each mutation reduces fitness more than the previous mutation. As such, the fitness function can be almost truncated at some number of mutations. At mutation-selection balance, the clonal population is stacked up against the fitness function, and the variance in mutation number is dramatically decreased. Then, during each bout of reproduction, some offspring obtain new mutations that essentially push them over the edge. In this highly simplified view of the model, sexual reproduction is favoured if the degree of synergism is sufficiently high (Kondrashov, 1988), and the rate of mutation per genome per generation is also sufficiently high (greater than 1–2; Howard & Lively, 1994). Recent empirical results are, however, inconsistent with both of these conditions (Keightley, 1996; de Visser, Hoekstra & Van Den Ende, 1997; Elena & Lenski, 1997b; Keightley & Eyre-Walker, 2000; Peters & Keightley, 2000).

EVOLUTION AND MAINTENANCE OF CLONAL DIVERSITY

Classical ecological theory leads to the expectation that the coexistence of clonal lineages is only possible if the clones are ecologically different (Gause, 1934; Vrijenhoek, 1978; Case & Taper, 1986). The diversity of clonal assemblages is increased by mechanisms that bring new clonal genotypes into the population, provided there is selection for rare clonal genotypes, and decreased by hard selection that favours the best-adapted clones.

GENERAL-PURPOSE GENOTYPE (GPG) MODEL

The 'general-purpose genotype model' (Baker, 1965; Lynch, 1984) suggests that those clones that best tolerate broad environmental conditions (i.e. have the highest geometric mean fitness) are the ones that persist in the long-run. The model assumes that clonal selection operates on an initially diverse clonal population in a habitat where the physical environment fluctuates in time. In such environments, some specialists become extinct each time the state of the environment shifts to unfavourable. The GPG model predicts that clonal assemblages are characterized by low clonal diversity, and that the same clonal lineages perform the best across a wide range of environments.

The GPG model is appealing because it is easy to accept that clonal selection would operate in natural environments as predicted by the model. Even so, in empirical studies, unambiguous support for the model has been difficult to find (Bierzychudek, 1989; Lynch, Spitze & Crease, 1989; Michaels & Bazzaz, 1989; Weider, 1993; Niklasson & Parker, 1994), although in some more recent studies support for the model is stronger (Jacobsen & Forbes, 1997; Jaenike & Dombeck, 1998; Schlosser *et al.*, 1998; Myers, Meyer & Resh, 2000), indicating that the GPG model may be an appropriate description of evolution of clonal assemblages in some specific systems.

FROZEN-NICHE VARIATION (FNV) MODEL

The 'frozen-niche variation model' (Vrijenhoek, 1979, 1984) proposes that clones in diverse populations are ecologically specialized, and express non-overlapping phenotypic variation. The model can be divided in two phases. Initially, clones originate from a sexual population, with each clone preserving a particular genotype and a corresponding slice of phenotypic variation of the original sexual population. Thus the first prediction of the model is that each clone has a lower phenotypic variance than the ancestral sexual population, i.e. none of the clones are omnipotent 'general-purpose genotypes'. During the second stage, clones compete

with the sexual population, replacing the overlapping sexual phenotypes. Clones also compete with each other, leading to selection that is expected to arrange clones into phenotypically non-overlapping arrays. The FNV model thus predicts that clones form stable, genotypically diverse populations where the fitnesses of the clones are equal at equilibrium. This prediction is in striking contrast with the predictions of the GPG model.

Several studies have addressed the FNV model, and found support for the first stage of the process (Paulissen, Walker & Cordes, 1988; Niklasson & Parker, 1994; Jokela *et al.*, 1997b; Semlitsch, Hotz & Guex, 1997; Weeks & Hoffmann, 1998; Gray & Weeks, 2001). Therefore, it seems to be common that clones have different phenotypes and different ecological requirements on which clonal selection can work. The FNV model is a plausible explanation for the spatial structure of clonal assemblages found in some systems (Weeks & Hoffmann, 1998), although even in uniform spatial mixtures of clones some aspect of their ecology might differ (Vrijenhoek, 1984; Wetherington *et al.*, 1989; Semlitsch, 1993). The second stage of the model (arrangement of clones in ecological arrays by competitive interactions) has also received some support (Semlitsch *et al.*, 1997; Weeks & Hoffmann, 1998).

PARASITE-MEDIATED FREQUENCY-DEPENDENT SELECTION

The power of negative frequency-dependent selection (FDS), i.e. 'rare advantage' in maintaining polymorphism is well known (see Hedrick, Ginevan & Ewing, 1976). If a rare genotype (or morph) has a fitness advantage, it will increase in frequency until it becomes common. As the genotype becomes common, its fitness advantage will erode and it will decline in frequency until it becomes rare. This process can lead to cycles in genotype frequencies, if selection is lagged in time. Such cycles are predicted, for example, when coevolving parasites track the common host genotypes (see above, 'the parasite hypothesis' for maintenance of sex; Jaenike, 1978; Hamilton, 1980; Hamilton *et al.*, 1990; Lively, Craddock & Vrijenhoek, 1990; Lively, 1999).

When applied to clonal assemblages, the parasite-mediated FDS model predicts that diverse populations of clones may coexist, as long as the clones differ in their resistance genotypes or by some other trait that is under negative frequency-dependent selection. The model predicts temporal dynamics in the clonal composition, where the most common clones alternate depending on the differences in the relative parasite pressure, or on fluctuating selection. FDS has been suggested to promote polymorphism in several empirical systems, many of which are not limited to coevo-

lutionary interactions with parasites or pathogens (Antonovics & Ellstrand, 1984; Pfennig, 1992; Hori, 1993; Roff, 1994; Benkman, 1996; Clay & Kover, 1996; Sinervo & Lively, 1996; Elena & Lenski, 1997a; Little & Ebert, 2001).

THE STUDY SYSTEM: *POTAMOPYRGUS ANTIPODARUM*

NATURAL HISTORY

Potamopyrgus antipodarum (Gray) is a freshwater prosobranch snail common in all sub-alpine freshwater habitats of New Zealand. It dominates the benthic invertebrate community in many different populations (Forsyth & McCallum, 1981; Gonzalez *et al.*, 1981; Biggs & Malthus, 1982; Timms, 1982; Michalkiewicz, 1991; Collier, Wilcock & Meredith, 1998), and may reach very high densities (up to 10⁶ individuals m⁻²; Ribi & Arter, 1986; Dorgelo, 1987; Schreiber *et al.*, 1998). Females brood offspring to a late stage of development (crawl-away larva) and reproduce throughout their lives (Townes, 1981). In captivity, snails may live several years (Wallace, 1992), but in natural populations most individuals are unlikely to live more than two summer seasons. Snails reach maturity within a few months in favourable conditions (Frenzel, 1979). Natural populations may therefore go through two to three generations per year (Frenzel, 1979, 1980).

MIXED POPULATIONS

The snail is special in that mixed populations of diploid sexual and triploid parthenogenetic individuals are common (Wallace, 1992; Dybdahl & Lively, 1995a), and that the frequency of sexual individuals in mixed populations varies from zero to almost 100% (Lively, 1987, 1992; Lively & Jokela, 2002). Conveniently, the proportion of males can be used to infer the proportion of diploid females in the population (Fig. 1). In addition, the frequency of sexual individuals may even vary between depth-specific habitat zones of the same population (Jokela & Lively, 1995a; Jokela *et al.*, 1997a). For example, in Lake Alexandrina the shallow shore-bank inhabiting population is highly sexual (up to 70–90%), while the deeper macrophyte inhabiting population is almost all clonal (<25% sexuals, Fig. 1, Jokela & Lively, 1995a; Jokela *et al.*, 1997a).

It is not known how triploid parthenogenetic clones are generated, but the most likely explanation is that an unreduced diploid gamete that is produced by disrupted meiosis is fertilized by a haploid gamete (Wallace, 1992; Dybdahl & Lively, 1995a). Parthenogenesis induced by the fertilization of unreduced eggs has been reported to lead to high levels of clonal diversity in weevils (Stenberg *et al.*, 2000).

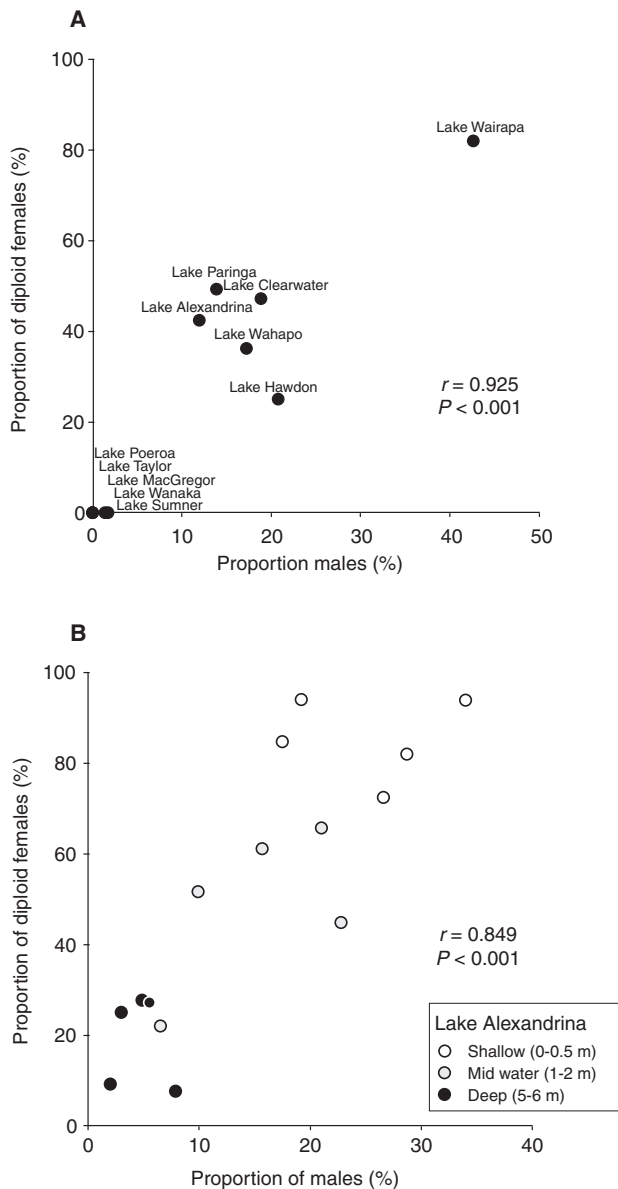


Figure 1. Proportion of diploid females plotted against the proportion of males in field populations of *Potamopyrgus antipodarum*. (A) The relationship between male frequency and the frequency of diploid females among New Zealand lakes. (B) The relationship between male frequency and the frequency of diploid females among three depth-specific habitats in Lake Alexandrina.

In all studied populations of *P. antipodarum*, clonal populations share almost all alleles (even rare ones) of the sympatric sexual population, indicating that the clones originated from the local sexual population (Dybdahl & Lively, 1995a; Fox *et al.*, 1996). Clones of the snail do not appear to be interspecific hybrids, as is the case for most asexual vertebrates (Vrijenhoek,

1990). In addition, the switch to parthenogenesis does not include genome duplication, which is the case for some invertebrate parthenogens (Bullini, 1965; Pertoldi, Scali & Loeschcke, 2001). It is important to note that parthenogenesis through chromosomally unreduced eggs does not dramatically change the degree of heterozygosity from that of the sexual population, which should be important for expectations of ecological similarity between sexuals and newly emerged parthenogens (Vrijenhoek & Lerman, 1982; Vrijenhoek, 1990).

Triploid clonal individuals are phenotypically indistinguishable from diploid sexual ones (Jokela *et al.*, 1997a). However, we have successfully used cellulose acetate gel electrophoresis (Richardson, Baverstock & Adams, 1986) to study population genetic and clonal structure, and to distinguish clonal individuals from the sexuals (e.g. Dybdahl & Lively, 1995a; Dybdahl & Lively, 1996). Electrophoresis is a convenient method because *P. antipodarum* populations are usually polymorphic at several loci, and because triploid heterozygotes can be directly inferred. In addition, because of this multilocus polymorphism, the resolution for identifying clonal genotypes is very high. For example, Fox (1995) estimated that, given the polymorphism present at six loci used in studies of snail populations from Lake Alexandrina, it would be theoretically possible to identify 45 000 multilocus genotypes in that population. More recently, microsatellite markers have become available (Weetman, Hauser & Carvalho, 2001).

Genotypic diversity of the clonal populations is high. For example, in a study of Lake Alexandrina, we found 165 clonal genotypes in the 605 individuals scored for six allozyme loci (Fox *et al.*, 1996). In other words, 27 different genotypes were detected per 100 triploid individuals, a very high degree of clonal diversity. Note that Lake Alexandrina has a mixed population of clones and sexuals, so if clones are of local origin, high diversity may not be that surprising. Yet clonal diversity may be very high even in predominately asexual populations. Thus in a recent study of Lake Tennyson (whose populations are <1% sexual), 59 different clonal genotypes were found among 561 scored individuals (11 clones per 100 triploid individuals; Jokela, Dybdahl & Lively, 1999). Furthermore, in both Lakes Alexandrina and Tennyson, clonal populations are structured by littoral habitat zones, suggesting that many such populations are habitat-specific.

TREMATODE PARASITES

Like many other gastropods, *P. antipodarum* harbours a rich community of harmful parasites, especially digenetic trematodes. These parasites have a complex life cycle, whereupon snails serve as intermediate

hosts. The definitive hosts are usually vertebrates, most often waterfowl and fish. More than 14 types (species) have been identified as infecting *P. antipodarum* (McFarlane, 1945; Winterbourn, 1974; Bisset, 1977; Jokela & Lively, 1995b), and all of these types usually sterilize infected individuals.

The most common of these parasites is an undescribed species of genus *Microphallus* (S. Deblock, pers. comm.). Susceptibility to *Microphallus* appears to vary among genotypes (Dybdahl & Lively, 1995b; Jokela *et al.*, 1997b), and both juveniles and adults have been found to be susceptible to infection in laboratory experiments (Krist & Lively, 1998; Krist *et al.*, 2000). The parasites have been found to be locally adapted to their host populations in several independent experiments (Lively, 1989; Lively & Dybdahl, 2000), suggesting that strong, local coevolutionary interactions between host and parasite are likely. The risk of trematode infection in the wild will obviously be affected by the distribution of final hosts (Jokela & Lively, 1995b), which in the case of *Microphallus* are dabbling ducks (Lively & McKenzie, 1991).

COST OF MALES IN *P. ANTIPODARUM*

If the clones do not have a faster intrinsic growth rate (r) than sexuals, then the whole issue of maintenance of sex becomes a 'red herring' (Jokela *et al.*, 1997a). The transition from sexual to asexual reproduction often involves genomic rearrangement, e.g. hybridization, increase in ploidy (Bell, 1982), which may directly interfere with the expression of fitness-related traits in the newly created asexual genotypes. For example, interspecific hybridization may lead to ecological differences between the ancestral sexual populations and the new clonal lineages (Wetherington *et al.*, 1989; Case, 1990). Similarly, the transition to parthenogenesis may lead to low viability of the asexuals (Corley & Moore, 1999; Innes, Fox & Winsor, 2000; Corley, Blankenship & Moore, 2001). Precisely because the transition to asexual reproduction can occur in many different ways, it is important in each case to test the assumption that the coexisting sexual and asexual lineages are ecologically similar. Verifying whether or not there is a cost of males (or a cost of sex, in general) is a fundamental step.

In an earlier study, we tested the 'all-else-equal' assumption by comparing phenotypic differences in life-history traits of clonal and sexual snails in Lake Alexandrina (Jokela *et al.*, 1997a). We found that snails from different depth-specific ecological zones expressed considerable phenotypic variation, but within each zone the average phenotype of the asexual population was indistinguishable from that of the sexuals. We combined this field study with a laboratory experiment where sexual populations were challenged

by one of the common coexisting clones. In all our replicate tanks, the growth rate of the clone was much higher than that of the sexual population (Jokela *et al.*, 1997a). The results of this one-year experiment suggest that this clone should be able to eliminate the sexual population (Jokela *et al.*, 1997a). Based on these results, we would expect rapid exclusion of sexual populations in the wild. To test this idea, we compared male frequencies in samples collected in the mid-1980s with male frequencies from samples collected in the late 1990s and 2000 from 20 natural lake populations. These lakes were chosen to represent the full range of variation in male frequency. We found that male frequencies changed, but not as dramatically as expected based on theory (Lively & Jokela, 2002). In fact, in most of the lakes, the male frequency changed very little over this time period, indicating that clones in these lakes are not replacing sexuals. Hence, some ecological or genetic factor (or a combination of the two) must be operating to select for sexual reproduction in the short-term.

THE MAINTENANCE OF SEX IN *P. ANTIPODARUM*

ECOLOGICAL MODELS

Potamopyrgus antipodarum is ideal for contrasting different hypotheses for maintenance of sex. Populations are found across a wide variety of environments, population sizes vary and, most importantly, the proportion of sexual individuals within populations varies. Studies conducted on this system during the past 15 years have focused primarily on ecological correlates of sex (but see Lively *et al.*, 1998). Earlier studies on this system contrasted the 'tangled bank hypothesis' (competitive interactions favour sex, e.g. Bell, 1982), the 'lottery hypothesis' (temporally unpredictable environments favour sex, e.g. Williams, 1975; Glesener & Tilman, 1978), and the 'reproductive assurance hypothesis' (Baker, 1965; Williams, 1975). No support was found for either the temporal variation hypothesis or the reproductive assurance hypothesis (Lively, 1987, 1992). The proportion of sexual individuals was higher in the presumably more highly competitive environments (e.g. lakes) as predicted by the tangled bank hypothesis, but more of the variation in male frequency was explained by the prevalence of infection by digenetic trematodes, suggesting that parasitism is more influential than habitat *per se* (Lively, 1987). Early studies therefore indicated that parasites were a more promising avenue for study than either competition in a spatially variable environment or temporal variation in the physical environment. This of course does not rule out the possibility that these processes have some influence.

In another field study, we unexpectedly encountered additional evidence in favour of the parasite model for sex. We found a positive and significant association between sex and prevalence of infection within a single lake. Specifically, we found that the shallow-water habitats of one lake (Lake Alexandrina) were occupied by highly sexual (>70% sexuals) populations of *P. antipodarum*, while the deeper littoral zones (>4 m) were largely clonal (<25% sexuals) (see Fig. 1B). Lake Alexandrina is homeothermic due to strong winds (Ward & Talbot, 1984), and no obvious environmental clines by depth exist that could be readily used to explain the observed cline in the proportion of sexual snails. However, we also found a cline for depth in the prevalence of infection (Jokela & Lively, 1995a). Shallow habitats had higher prevalence of infection than deep habitats, and prevalence of infection was a significant predictor of the proportion of sexual individuals in the sample (Jokela & Lively, 1995a).

The association between the prevalence of infection and proportion of sexual individuals is consistent with one fundamental expectation of the parasite hypothesis for sex. The advantage to sexuals should be higher in coevolutionary hotspots, where parasite pressure is highest, and while these studies are not a proof of the parasite hypothesis, they could easily have rejected it (Lively, 2001).

MUTATION MODELS

The mutational deterministic model (MD) is especially attractive because of its clear predictions which allow empirical testing. We addressed the MD model for maintenance of sex in *P. antipodarum* with an ecological experiment (Lively *et al.*, 1998). The experiment relies on the prediction that mutation load at mutation-selection balance should respond to variation in the harshness of the environment (Kondrashov, 1995). Under harsh conditions fewer mutations suffice to generate the same reduction in fitness as a higher mutation load under favourable conditions. Note that the MD model predicts a different shape for the distribution of mutations at mutation-selection balance for clones and sexuals: clones should be narrowly stacked against the fitness function close to mutation-selection balance, while sexuals should have more low mutation classes (Fig. 2A). If environmental harshness is experimentally increased, then mutation-selection balance should be met at a lower mutation load (Fig. 2B). As this happens, more clones than sexuals should be pushed over the line (to the 'wrong' side of the fitness function). Our prediction was that if sex is maintained under the MD model in *P. antipodarum*, clones should suffer more under severe starvation stress than sexuals, and we should observe a decrease in the proportion of clonal individuals (Lively *et al.*,

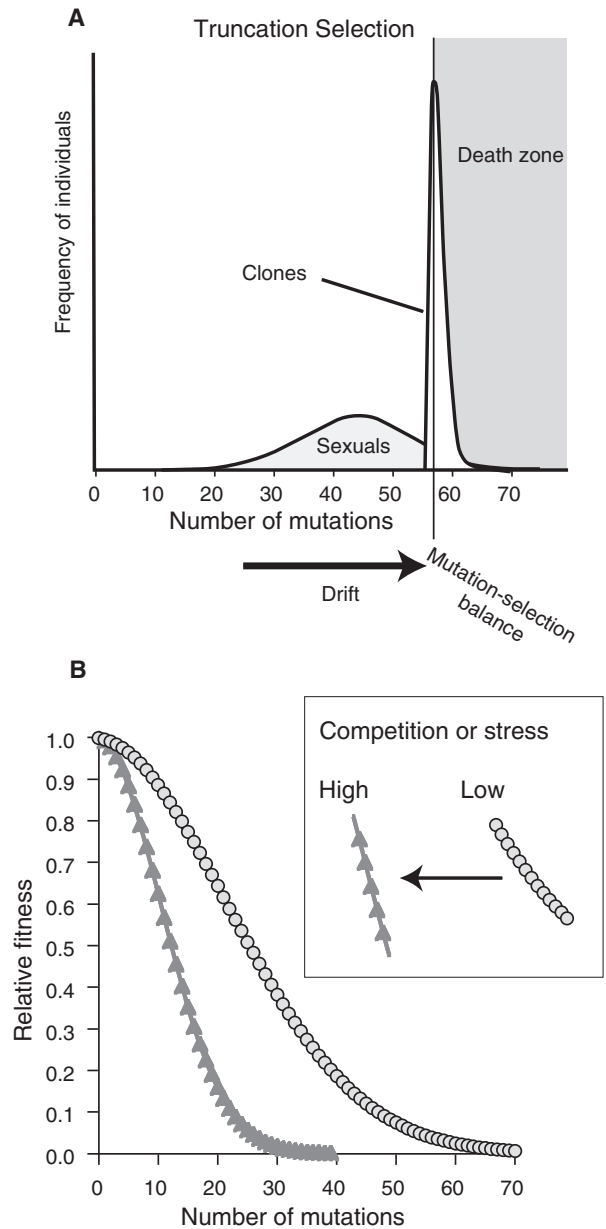


Figure 2. (A) Distribution of mutations in sexual and clonal population when populations have reached mutation-selection balance under truncation selection (example of extreme synergism). Note that the clonal population has much narrower distribution of mutation classes than the sexual population because sexuals can regenerate low mutation classes through recombination. Vertical line depicts the truncation point at which more mutations lead to death. (B) Synergistic fitness functions are expected to respond to environmental stress. Mutation-selection balance is expected to be found at lower mutation load when stress increases. (Fig. 2B reproduced from Lively *et al.*, 1998.)

1998). We related this prediction to the cline by depth in the proportion of sexuals in Lake Alexandrina. More specifically, if the cline were due to habitat-specific variation in stress, then snails in the middle zone should sort out by their reproductive mode in response to stress.

We designed an experiment in which we exposed replicate containers of snails from the middle zone of Lake Alexandrina (50% sexuals) to different levels of stress along a starvation gradient (Lively *et al.*, 1998). We reasoned that starvation is an expedient way to express differences in the mutation load between sexual and asexuals, provided the treatment is sufficiently severe. At the beginning of the experiment, we sampled the study population for the proportion of sexual individuals. We then ran the experiment, scoring the mortality and growth of individuals over the starvation gradient (Fig. 3A,B). Starvation was successful in increasing mortality and decreasing growth rate, but we did not observe any differential response in the proportion of sexual individuals (Lively *et al.*, 1998; Fig. 3). In other words, sexual and clonal snails had similar mortality rates. This result suggests that (1) either the clonal population was not in mutation-selection balance (perhaps due to a very recent origin), or (2) the synergism among mutations is weak or absent. Whatever the detailed explanation, the results suggest that MD theory is an insufficient explanation for maintenance of sex in *P. antipodarum*.

THE EVOLUTION AND MAINTENANCE OF CLONAL DIVERSITY IN *P. ANTIPODARUM*

FNV AND GPG IN *P. ANTIPODARUM*

We tested the FNV model in a mixed population of *P. antipodarum* (Jokela *et al.*, 1997b). We found that each clone expressed narrower phenotypic variation than the sexual population, supporting the first stage of the FNV model. It seems that each clone preserves a slice of the phenotypic variation present in the sexual population. However, several clones had completely overlapping distributions of phenotypic variation, and the combined phenotypic distribution of the clones completely overlapped that of the sexuals. These results therefore suggest that the clones preserve the phenotypic variation of the corresponding sexual genotype, but that clonal selection does not

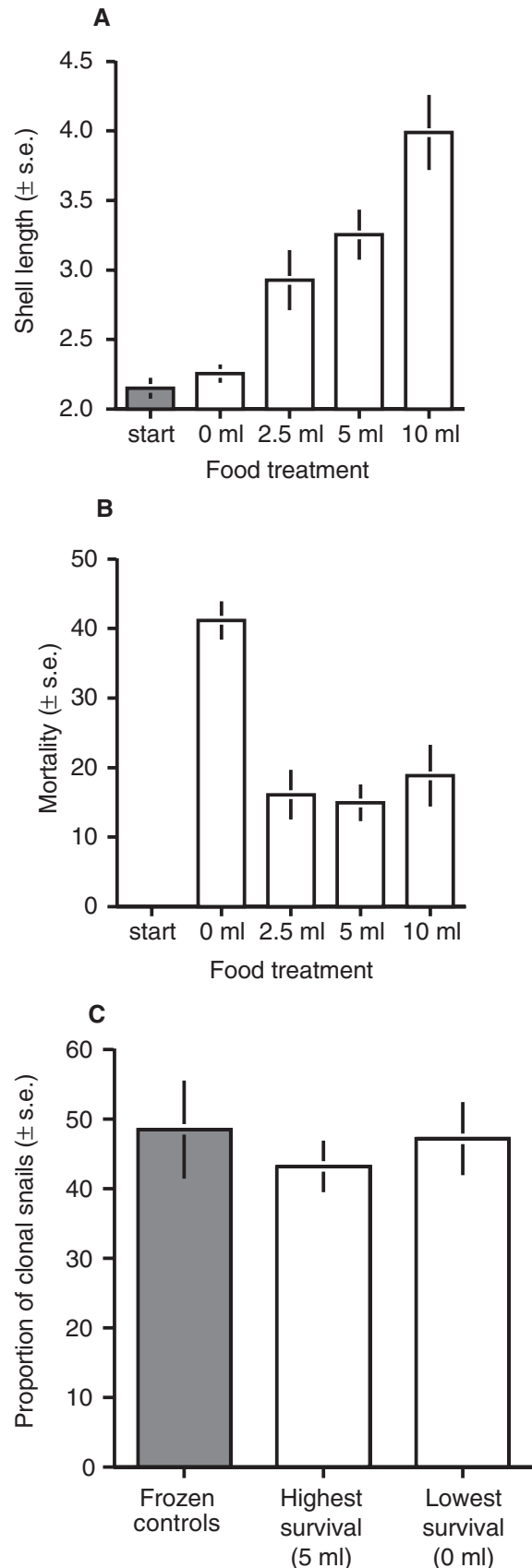


Figure 3. Response of mixed population of *P. antipodarum* to starvation. (A) Growth rate was markedly reduced across the food gradient. (B) Mortality was highest in the lowest food treatment. (C) The proportion of clonal snails did not respond to increased mortality. (Figure reproduced from Lively *et al.*, 1998.)

lead to arrangement of clones in phenotypic arrays, and that competition between the clones and the sexuals does not lead to exclusion of the sexual individuals in the mixed populations of these snails (Jokela *et al.*, 1997b).

Interestingly, the opposite conclusion was reached by Jacobsen & Forbes (1997) for the extant clones of *P. antipodarum* that were introduced to Europe about 100 years ago. In Europe, it appears that the remaining clones express the characteristics of the GPG model: wide tolerance of some genotypes over a range of environments, and consistent fitness rank of genotypes over an array of environments. The case may not be comparable to the native populations of New Zealand, because sexual populations are not found in Europe. In other words, no new clones have originated in Europe, and those clones that are left in Europe are those that have successfully persisted from the group of genotypes that were originally introduced. In such a scenario, selection is likely to sort out the most broadly tolerant genotypes of those available.

THE PARASITE HYPOTHESIS FOR CLONAL DIVERSITY

Most populations of *P. antipodarum* show a high level of clonal diversity (Dybdahl & Lively, 1995a,b; Fox *et al.*, 1996; Jokela *et al.*, 1999). The existence of such high diversity in mixed populations might be explained, in part, by mutational input from the sexual population. But the maintenance of high clonal diversity in all-female populations is truly vexing since several ecological and evolutionary processes should operate to erode diversity. Muller's ratchet would be expected to drive some clonal lineages extinct as they accumulated mutations. In addition, if selection favours 'general-purpose genotypes', these will displace specialist clones (Lynch, 1984). Lastly, interclonal selection may result in the rapid displacement of competitively inferior clones, or those with lower intrinsic growth rates.

Parasite-mediated frequency-dependent selection could inhibit some of this erosion of clonal diversity (Lively & Howard, 1994) because parasites are expected to produce an advantage to rare genotypes. Because this selection occurs with a time lag, the frequency of host genotypes might be expected to cycle (Hutson & Law, 1981; Bell, 1982). The consequence for clonal diversity is that long-term dominance by a single clone is prevented.

CLONAL DYNAMICS IN AN ALL FEMALE POPULATION

We have been examining the role of parasite-mediated selection in *P. antipodarum* using a clonal population in Lake Poerua on the South Island of New Zealand. We began a long-term study of coevolu-

tionary dynamics in this lake in 1992 by tracking the frequencies of clonal genotypes (using electrophoresis) and their levels of infection by digenetic trematodes (Dybdahl & Lively, 1998). Between 1992 and 1996, we found that four different clones (multilocus genotypes) dominated the population in different years. We also found that the infection rate increased in each of the four clones until they were significantly over-infected, and that three of these clones subsequently declined in frequency. In addition, this over-infection of common clones occurred with a time lag. We found a significant correlation between the change in the frequency of a clone and the change in its rate of infection one year later (Dybdahl & Lively, 1998).

It is of course possible that these field results were due to environmental influences on susceptibility (Little & Ebert, 2000), or to an inherently greater susceptibility of common clones. The common clones might have been more susceptible, for example, if they invested more resources in growth and reproduction than in defence against pathogens and parasites (the trade-off hypothesis). We tested these possibilities in laboratory infection experiments, and found that parasites from Lake Poerua were more infective to clones that had been common in the recent past than to clones that had been rare during the same period; hence the field results are unlikely to be strictly due to environmental influences (Dybdahl & Lively, 1998). In a follow-up experiment, we also found that these common clones were not more infectable than rare clones to parasites from a different lake, thereby falsifying the trade-off hypothesis (Lively & Dybdahl, 2000). Note that we do not claim that trade-offs do not exist, but rather that trade-offs are unlikely to explain the disproportionate infection of common clones in two separate experiments.

Strong antagonistic coevolution should also help maintain clonal diversity in the face of processes that might erode it. We examined the relative strengths of the processes that erode vs. maintain clonal diversity by comparing measures of diversity values over a 10-year period in Lake Poerua. Clonal diversity was measured as G_0 , which varies from a low of 1 to the total number of clones in the sample (Stoddard, 1983; Stoddard & Taylor, 1988). We also measured evenness, 'E', which is affected by the relative abundance of each clone, and it varies between 0 and 1 (Stoddard, 1983; Stoddard & Taylor, 1988). Overall, we found that clonal diversity and evenness in the Lake Poerua population were relatively high (Table 1) compared to other *P. antipodarum* populations (range: 2.2–8.2 for G_0 , and 0.45–0.92 for E; Dybdahl & Lively, 1995a,b), and these indices changed little over time. In fact, these indices have remained remarkably constant (Table 1). Hence, it would seem that some selective

Table 1. Indices of clonal diversity and evenness for Lake Poerua over a 10-year period. Clonal diversity was measured as G_0 , which varies from a value of 1 to the total number of clones in the sample, and as evenness, E (the relative abundance of each clone, which varies between 0 and 1)

Year	N	# clones	Clones per individual	G_0	E
1992	75	25	0.33	7.30	0.85
1993	58	20	0.34	9.09	0.90
1994	97	34	0.35	12.02	0.92
1995	96	22	0.23	7.72	0.89
1996	156	35	0.22	12.66	0.93
1997	129	39	0.30	14.16	0.93
1998	214	45	0.21	9.85	0.90
1999	216	47	0.22	9.86	0.90
2000	214	46	0.21	11.58	0.92
2001	229	54	0.24	10.54	0.90

force must have been operating in the wild to prevent the short-term elimination of clones.

THE DISTRIBUTION OF CLONAL DIVERSITY IN A MIXED POPULATION

From our earlier studies, we know that there is a cline in the frequency of sexual individuals in Lake Alexandrina such that there are more sexual individuals in shallow than in deep water (5–6 m. Jokela & Lively, 1995a; Fig. 1A). However, clonal females coexist with sexual females in both shallow and deep water. Here we ask: how does clonal diversity vary with depth?

There is one very good reason to suspect that clonal diversity might be higher in the shallow water habitat (where clonal diversity is defined as the number of different clonal genotypes divided by the number of clonal individuals sampled). The reason is that the shallow water habitat has a higher frequency of sexual snails, which is the ultimate source of clones. Another reason to expect higher clonal diversity is that the prevalence of infection (and presumably the risk of infection) is greater in the shallow water, suggesting that parasite-mediated selection against common genotypes might result in higher clonal diversity there. To determine the influences of parasites and the frequency of sexuals on local clonal diversity, we conducted a stepwise multiple regression using the proportion of sexuals and the frequency of infected snails as independent variables. We also included Nei's (1978) genetic diversity index (H) as an independent variable. This index gives a measure of the genetic diversity within the local sexual group, which

is potentially limiting to the diversity of clones generated from the group, assuming limited migration. Our use of the stepwise regression analysis was primarily to generate partial correlation coefficients rather than to assert causality.

The data we analysed came from the Fox *et al.* (1996) study. The data set consists of 15 samples of snails, five samples from each of the three depth-specific habitat zones (shallow, mid-water, and deep). Cellulose acetate electrophoresis was used to determine ploidy and multilocus genotype for each of about 100 snails at each site. The results were surprising. The proportion of sexual individuals explained most of the variance in clonal diversity, and it was significantly positively correlated with clonal diversity ($r = 0.668$; $P = 0.003$). Hence, there are more clonal genotypes per triploid individual where more diploid sexual individuals are living, which is in the shallow water habitat (Fig. 4A). The prevalence of infection by *Microphallus* was significantly correlated with the residuals, but the correlation was negative, rather than positive (partial $r = -0.629$; $P = 0.016$; Fig. 4B). In other words, samples showing positive residuals tended to be collected at sites with relatively low levels of infection and vice versa. These two independent variables (% sexuals and % infected) explained 61% of the total variation in clonal diversity (adjusted $R^2 = 0.61$, $F_{2,12} = 11.93$, $P = 0.001$). The direct measure of genetic diversity in the sexual population was not significantly correlated ($r = 0.373$; $P = 0.086$) or partially correlated with clonal diversity (partial $r = 0.172$, $P = 0.557$), and did not enter the final regression model.

These results suggest that the presence of sexual snails is more influential on clonal diversity than the presence of parasites. So we suggest that parasites might be maintaining sex in the shallow water, and that the sexuals are generating new clones leading to higher clonal diversity in the shallow water. The results also suggest that *Microphallus* infection may be a product of local clonal diversity, rather than the other way around. As such, these results support the hypothesis that infection is locally high where a few clones dominate. This might lead to higher clonal diversity at the level of the lake, or habitats within lakes, but not at the level of site within habitat. Unfortunately, the present data set is too small to analyse at the level of clonal genotype, but the idea is testable. In larger samples, we would expect under this hypothesis that if clonal genotypes that are widely distributed in the lake are also very common in some locations, the infection level would be higher than expected based on the frequency of diploid sexual snails in the same area.

Genetic diversity (H) was not a significant predictor of clonal diversity in the above analysis, but we nonetheless were interested in the distribution of genetic

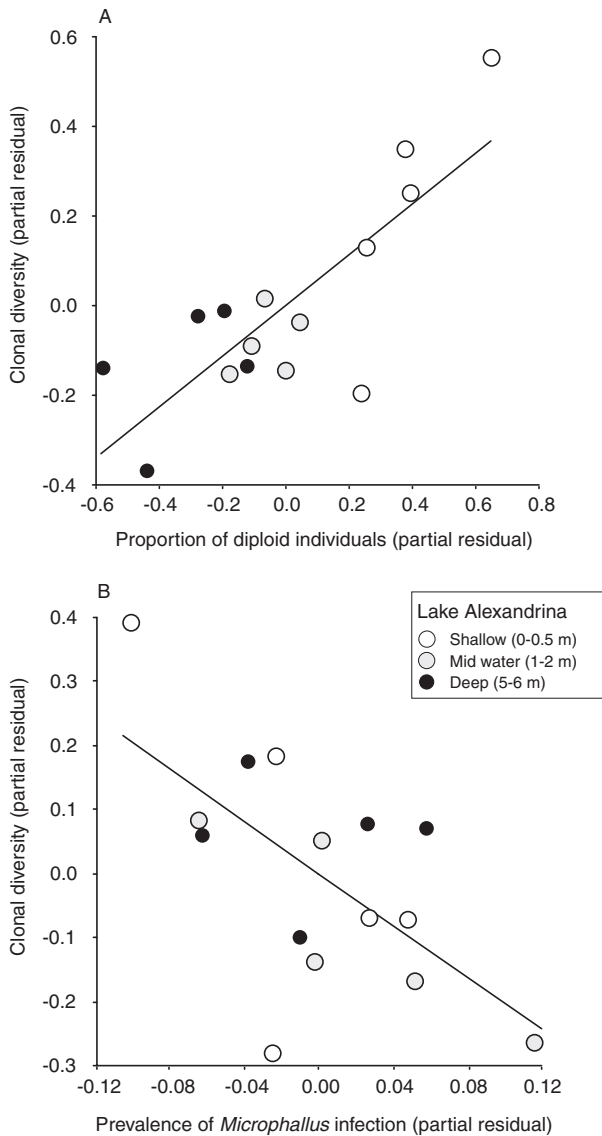


Figure 4. Association between clonal diversity (number of triploid genotypes/number of triploid individuals), proportion of diploid individuals (sexuals) and prevalence of infection in 15 samples from Lake Alexandrina (Fox *et al.*, 1996). (A) Clonal diversity increases with proportion of diploid individuals in the population, and (B) decreases as the prevalence of infection by *Microphallus* increases. Panels A and B present partial residuals of a multiple regression analysis (see text). Symbols indicate samples from three depth-specific habitat zones of the lake.

diversity within the sexual subpopulation. We first plotted the relationship between diversity of the sexual population and the proportion of sexual individuals in the three depth-specific habitat zones of Lake Alexandrina. We found that, in general, genetic diversity of sexuals varied relatively little among the 15

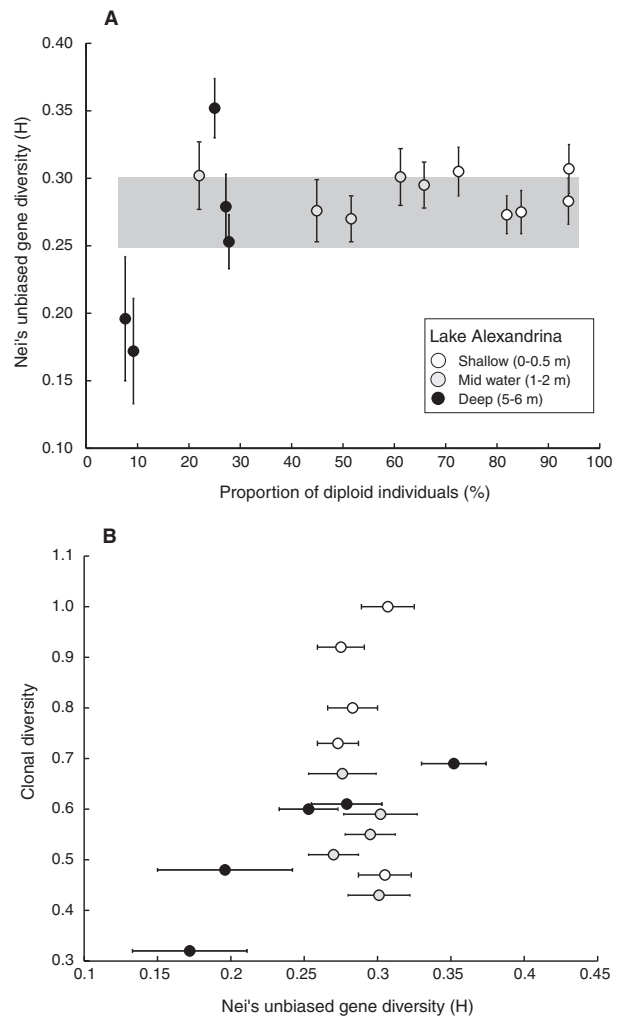


Figure 5. (A) Relationship between the proportion of diploid individuals (sexuals) and genetic diversity (± 1 bootstrapped SD) of the sexual population for samples from three depth-specific habitat zones of Lake Alexandrina. Gray area depicts the 95% confidence area for the mean genetic diversity. (B) Relationship between genetic diversity and clonal diversity (number of triploid multilocus genotypes per number of triploid individuals in the sample) in the same samples.

samples (Fig. 5A). However, as Figure 5A shows, diversity was the lowest in those two samples where the proportion of sexual individuals was also lowest (<10%). In samples where the proportion of sexuals was higher (>30%), genetic diversity varied only little and did not increase with the proportion of sexual individuals (Fig. 5B). These data argue that if sexuals become very rare, as in some deep sites of Lake Alexandrina, it is possible that the sexual population loses alleles due to inbreeding and drift. We also found that genetic diversity was very similar among shallow-

water and mid-water sites and uncorrelated with the clonal diversity at these sites (Fig. 5B). However, genetic diversity was highly variable among the deep-water sites and positively correlated with clonal diversity (Fig. 5B). Thus, genetic diversity of the local sexual population might limit clonal diversity in the deeper habitats but, overall, clonal diversity seems to be more dependent of the frequency of sexual individuals within habitats than on the allelic diversity of sexuals in these habitats.

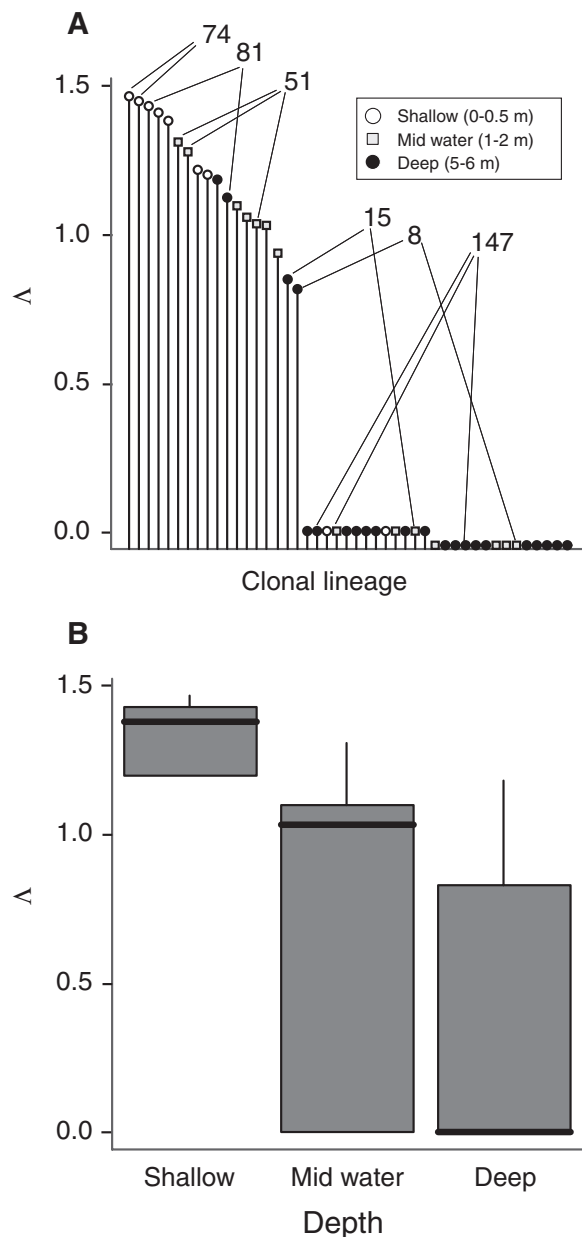
GENETIC BASIS OF LIFE-HISTORY VARIATION AMONG CLONES

Earlier work revealed consistent phenotypic differences in life-history traits among clones, suggesting that fitness might also vary (Jokela *et al.*, 1997b). In 1994, one of us (J.J.) isolated 45 females of Lake Alexandrina to study growth rate of clonal lineages in a controlled laboratory setting. These snails came from the three main littoral habitat zones of the lake (shallow, mid-water, and deep). Offspring of these snails were raised individually in standard 200 mL disposable party cups to estimate survival and fecundity for each lineage. Growth rates (λ) of these lineages were then estimated using standard Leslie-matrix methods (Caswell, 1989). Conditions during the 16-month experiment were kept constant. The experiment was designed to measure performance of these clones in an environment where the clones were free of ecological interactions with competitors or enemies.

Figure 6 illustrates the differences in the growth rates of these clonal lineages in the 'common garden'.

Figure 6. Growth rate (λ) of clonal *P. antipodarum* lineages that were isolated from Lake Alexandrina. Each lineage was started by a single female. Fecundity and survival of each offspring was used to calculate a life-table; λ was calculated from this table using a Leslie-matrix. The experiment lasted for 16 months during which life-history data for 422 offspring were recorded. (A) The estimated value of λ for 45 clonal lineages. The clones are ranked according to their growth rates. Symbols indicate the habitat from which the parental female was isolated. Values above 1 indicate positive population growth. A value of $\lambda = 0$ was given to lineages that did not produce any reproducing offspring in 16 months. λ was not estimated for the last 14 lineages, because offspring from these lineages did not survive more than 2 weeks. Lines above the symbols connect lineages that have the same multilocus genotype. Numbers for these genotypes are the same as used in Fox *et al.* (1996). (B) Box-plot of growth rate, λ , by habitat of origin. Black horizontal line indicates the median value for lineages in each habitat.

The results are surprising. Only 15 of the initial 45 lineages had population growth rates greater than one. This result suggests that although the clonal diversity may appear high, the 'effective diversity' (how many high-fitness lineages are in the population) may be lower. The data also shows that habitat source affects the clonal growth rate. Lineages from the shallow water habitat had higher population growth rates than lineages from the deep water (Kruskal–Wallis test, asymptotic $P = 0.007$; Fig. 6B). This may reflect habitat-specific life-history differences in the ancestral sexual population from which these clones were derived. Finally, since the original 45 lineages were



randomly chosen among the individuals from each habitat, some of the parental snails were of the same multilocus genotype, replicating particular clonal genotypes in the experiment (Fig. 6A). For these six replicated clonal lines, the variation among lines was higher than variation within lines (Kruskal–Wallis test, exact $P = 0.007$). These results indicate, perhaps not surprisingly, that the clones are ‘not equal’ with respect to their life-history traits, and that important parts of variation in their life-history traits have a genetic basis.

SUMMARY

Most hypotheses for the maintenance of sexual reproduction have assumed that the benefits of outcrossing stem directly from the genetic diversity conferred by meiosis and syngamy on outcrossed progeny (but see Bernstein *et al.*, 1985). However, the benefit of producing variable progeny is likely to be reduced under each of these hypotheses whenever sexual individuals coexist with a genetically diverse population of asexual individuals. Therefore, the maintenance of sex in organisms like *P. antipodarum*, for which we have found a high degree of clonal diversity, is particularly intriguing.

The solution for the maintenance of sex in these snails, and in general, is clearly not at hand, but the study of *P. antipodarum* and other systems has led to a sorting out of hypotheses. In particular, the ‘lottery model’, despite its conceptual appeal, has not been supported by our data. The model is also in conflict with the massive amount of data assembled in Bell’s (1982) review of the distribution of parthenogenesis. Similarly, the reproductive assurance hypothesis is not supported by our data and although it might explain the evolution of uniparental reproduction in some species, the hypothesis nonetheless fails in the sense that it does not explain the maintenance of sex in those areas where finding mates is not difficult.

The importance of soft selection (the ‘tangled bank’/‘frozen niche variation’ hypotheses) is more difficult to ascertain. Our results suggest that soft selection is probably not important to the maintenance of sex in *P. antipodarum*, because sexuals and asexuals coexist within the depth-stratified habitats in Lake Alexandrina. We would agree that the possibility exists that there are microhabitats within each of these areas, upon which the many different clones might specialize, but the existence of these microhabitats is not obvious to us at the present time. Soft selection might, however, play some role in the distribution of clones among habitats in both Lakes Alexandrina and Tennyson, but recent reciprocal transplant experiments have suggested that clones may be quite plastic with respect to habitat use (Negovetic & Jokela, 2001).

In contrast, the ‘parasite hypothesis’ has been surprisingly robust in the face of results from field surveys and laboratory experiments. Firstly, parasite prevalence is the best predictor we presently have of the presence of males in natural populations. This result holds both among populations across New Zealand, and within one lake (Alexandrina) where a cline in the frequency of males is known to exist. Furthermore, these results have been recently shown to be repeatable over time at both spatial scales. We accept that ‘correlation is not causation’, and we do not present these findings as definite. Nonetheless, the correlations are surprising and interesting. Secondly, we have found a strong rare advantage in experimental exposures to parasites: rare clones were more resistant to infection in two different experiments. In addition, field data on this same population (Lake Poerua) have shown patterns in the rise and fall of common clonal genotypes that seem to be uniquely predicted by the parasite hypothesis.

With these results in mind, however, it would seem to us that the parasite hypothesis is not a sufficient explanation for the maintenance of sex in *P. antipodarum* or any other organism, since it relies on frequency-dependent selection. However, parasite coevolution and the accumulation of mutations by Muller’s ratchet could act together to prevent the accumulation of clonal diversity and the eventual elimination of sex (Howard, 1994; Howard & Lively, 1998; West, Lively & Read, 1999).

ACKNOWLEDGEMENTS

We thank P. Mutikainen and two anonymous reviewers for comments on the manuscript. These studies were made possible by the continuous support of the Edward Percival Field Station, the University of Canterbury’s Zoology Department, Jack van Berkel and Jan McKenzie. Our work was funded by the Academy of Finland (J.J.), the Swiss National Science Foundation (J.J.), the Marsden Fund (C.M.L.) and the US National Science Foundation (DEB 0128510 to J.J. and C.M.L., DEB 9904840 to C.M.L.; DEB 9907373 to M.F.D.).

REFERENCES

- Antonovics J, Ellstrand NC. 1984.** Experimental studies of the evolutionary significance of sexual reproduction. I. A test of the frequency-dependent selection hypothesis. *Evolution* **38**: 103–115.
- Baker HG. 1955.** Self-compatibility and establishment after ‘long-distance’ dispersal. *Evolution* **9**: 347–348.
- Baker HG. 1965.** Characteristics and models of origin of weeds. In: Baker HG, Stebbins GI, eds. *Genetics of colonizing species*. New York: Academic Press, 137–172.

- Bell G. 1982.** *The masterpiece of nature: the evolution and genetics of sexuality*. Berkeley, CA: University of California Press.
- Bell G. 1988a.** Recombination and the immortality of the germ line. *Journal of Evolutionary Biology* **1**: 67–82.
- Bell G. 1988b.** *Sex and death in protozoa: the history of an obsession*. Cambridge: Cambridge University Press.
- Benkman CW. 1996.** Are the ratios of bill crossing morphs in crossbills the result of frequency-dependent selection? *Evolutionary Ecology* **10**: 119–126.
- Bernstein H, Byerly HC, Hopf FA, Michod RE. 1985.** Genetic damage, mutation, and the evolution of sex. *Science* **229**: 1277–1281.
- Bierzychudek P. 1989.** Environmental sensitivity of sexual and apomictic Antennaria: do apomicts have general purpose genotypes? *Evolution* **43**: 1456–1466.
- Biggs BJF, Malthus TJ. 1982.** Macroinvertebrates associated with various aquatic macrophytes in the backwaters and lakes of the upper Clutha Valley, New Zealand. *New Zealand Journal of Marine and Freshwater Research* **16**: 81–88.
- Bisset SA. 1977.** *Notocotylus tadornae* n.sp. and *Notocotylus gippyensis* (Beverley-Burton, 1958) (Trematoda: Notocotylidae) from waterfowl in New Zealand: morphology, life history, and systematic relations. *Journal of Helminthology* **51**: 365–372.
- Bullini L. 1965.** Research into the biological characteristics of amphigony and parthenogenesis in a bisexual population of *Bacillus rossius* (Rossi) (Cheleutoptera, Phasmoidea). *Rivista di Biologia* **58**: 189–244.
- Case TJ. 1990.** Patterns of coexistence in sexual and asexual species of *Cnemidophorus* lizards. *Oecologia* **83**: 220–227.
- Case TJ, Taper ML. 1986.** On the coexistence of asexual and sexual species. *Evolution* **40**: 366–387.
- Caswell H. 1989.** *Matrix population models*. Sunderland, MA: Sinauer Associates Inc.
- Chao L. 1990.** Fitness of RNA virus decreased by Muller's ratchet. *Nature* **348**: 454–455.
- Clarke B. 1976.** The ecological relationships of host-parasite relationships. In: Taylor AER, Muller R, eds. *Genetic aspects of host-parasite relationships*. Oxford: Blackwell Scientific, 87–103.
- Clay K, Kover PX. 1996.** The Red Queen hypothesis and plant-pathogen interactions. *Annual Review of Phytopathology* **34**: 29–50.
- Collier KJ, Wilcock RJ, Meredith AS. 1998.** Influence of substrate type and physico-chemical conditions on macroinvertebrate faunas and biotic indices of some lowland Waikato, New Zealand, streams. *New Zealand Journal of Marine and Freshwater Research* **32**: 1–19.
- Corley LS, Blankenship JR, Moore AJ. 2001.** Genetic variation and asexual reproduction in the facultatively parthenogenetic cockroach *Nauphoeta cinerea*: implications for the evolution of sex. *Journal of Evolutionary Biology* **14**: 68–74.
- Corley LS, Moore AJ. 1999.** Fitness of alternative modes of reproduction: Developmental constraints and the evolutionary maintenance of sex. *Proceedings of the Royal Society of London B* **266**: 471–476.
- Dorgelo J. 1987.** Density fluctuations in populations (1982–86) and biological observations of *Potamopyrgus jenkinsi* in two trophically differing lakes. *Hydrobiological Bulletin* **21**: 95–110.
- Dybdahl MF, Lively CM. 1995a.** Diverse endemic and polyphyletic clones in mixed populations of the freshwater snail, *Potamopyrgus antipodarum*. *Journal of Evolutionary Biology* **8**: 385–398.
- Dybdahl MF, Lively CM. 1995b.** Host–parasite interactions: infection of common clones in natural populations of a freshwater snail (*Potamopyrgus antipodarum*). *Proceedings of the Royal Society of London B* **260**: 99–103.
- Dybdahl MF, Lively CM. 1996.** The geography of coevolution: Comparative population structures for a snail and its trematode parasite. *Evolution* **50**: 2264–2275.
- Dybdahl MF, Lively CM. 1998.** Host–parasite coevolution: Evidence for rare advantage and time-lagged selection in a natural population. *Evolution* **52**: 1057–1066.
- Elena SF, Lenski RE. 1997a.** Long-term experimental evolution in *Escherichia coli*. VII. Mechanisms maintaining genetic variability within populations. *Evolution* **51**: 1058–1067.
- Elena SF, Lenski RE. 1997b.** Test of synergistic interactions among deleterious mutations in bacteria. *Nature* **390**: 395–398.
- Felsenstein J. 1974.** The evolutionary advantage of recombination. *Genetics* **78**: 737–756.
- Forsyth DJ, McCallum ID. 1981.** Benthic macroinvertebrates of Lake Taupo, New Zealand. *New Zealand Journal of Marine and Freshwater Research* **15**: 41–46.
- Fox JA. 1995.** The diversity and distribution of clones and sexuals across habitats in a mixed population of a freshwater snail (*Potamopyrgus antipodarum*). Unpublished MSc Thesis. Department of Biology. Bloomington: Indiana University.
- Fox JA, Dybdahl MF, Jokela J, Lively CM. 1996.** Genetic structure of coexisting sexual and clonal subpopulations in a freshwater snail (*Potamopyrgus antipodarum*). *Evolution* **50**: 1541–1548.
- Frenzel P. 1979.** Biology and population dynamics of *Potamopyrgus jenkinsi* (Gastropoda: Prosobranchia) in the littoral of Lake Constance, West Germany. *Archiv für Hydrobiologie* **85**: 448–464.
- Frenzel P. 1980.** Production of *Potamopyrgus jenkinsi* (Gastropoda, Prosobranchia) in Lake Constance. *Hydrobiologia* **74**: 141–144.
- Gause GF. 1934.** *The struggle for existence*. New York: Dover.
- Ghiselin MT. 1974.** *The economy of nature and the evolution of sex*. Berkeley, CA: University of California Press.
- Glesener RR, Tilman D. 1978.** Sexuality and the components of environmental uncertainty: clues from the geographic parthenogenesis in terrestrial animals. *American Naturalist* **112**: 659–673.
- Gonzalez G, Tort MJ, Prat N, Puig MA. 1981.** Distribution of *Potamopyrgus jenkinsi* (Gastropoda, Hydrobiidae) in the basin of Besos and Llobregat rivers (northeastern Spain). *Iberus* **1**: 61–66.
- Gray MM, Weeks SC. 2001.** Niche breadth in clonal and sexual fish (*Poeciliopsis*): a test of the frozen niche variation

- model. *Canadian Journal of Fisheries and Aquatic Sciences* **58**: 1313–1318.
- Haigh J. 1978.** The accumulation of deleterious genes in a population: Muller's ratchet. *Theoretical Population Biology* **14**: 251–267.
- Haldane JBS. 1949.** Disease and evolution. *La Ricerca Scientifica (Supplement)* **19**: 68–76.
- Hamilton WD. 1980.** Sex versus non-sex versus parasite. *Oikos* **35**: 282–290.
- Hamilton WJ. 1982.** Pathogens as causes of genetic diversity in their host populations. In: Anderson RM, May RM, eds. *Population biology of infectious diseases*. New York: Springer-Verlag, 269–296.
- Hamilton WD, Axelrod R, Tanese R. 1990.** Sexual reproduction as an adaptation to resist parasites (a review). *Proceedings of the National Academy of Sciences, USA* **87**: 3566–3573.
- Hedrick PW, Ginevan ME, Ewing EP. 1976.** Genetic polymorphism in heterogeneous environments. *Annual Review of Ecology and Systematics* **7**: 1–32.
- Hori M. 1993.** Frequency-dependent natural selection in the handedness of scale-eating cichlid fish. *Science* **260**: 216–219.
- Howard RS. 1994.** Selection against deleterious mutations and the maintenance of biparental sex. *Theoretical Population Biology* **45**: 313–323.
- Howard RS, Lively CM. 1994.** Parasitism, mutation accumulation and the maintenance of sex. *Nature* **367**: 554–557.
- Howard RS, Lively CM. 1998.** The maintenance of sex by parasitism and mutation accumulation under epistatic fitness functions. *Evolution* **52**: 604–610.
- Howard RS, Lively CM. 2002.** The ratchet and the Red Queen: the maintenance of sex in parasites. *Journal of Evolutionary Biology* **52**: 648–656.
- Hutson V, Law R. 1981.** Evolution of recombination in populations experiencing frequency-dependent selection with time delay. *Proceedings of the Royal Society Series B, Biological Sciences* **213**: 345–359.
- Innes DJ, Fox CJ, Winsor GL. 2000.** Avoiding the cost of males in obligately asexual *Daphnia pulex* (Leydig). *Proceedings of the Royal Society of London B* **267**: 991–997.
- Jacobsen R, Forbes VE. 1997.** Clonal variation in life history traits and feeding rates in the gastropod, *Potamopyrgus antipodarum*: performance across a salinity gradient. *Functional Ecology* **11**: 260–267.
- Jaenike J. 1978.** An hypothesis to account for the maintenance of sex in populations. *Evolutionary Theory* **3**: 191–194.
- Jaenike J, Dombeck I. 1998.** General-purpose genotypes for host species utilization in a nematode parasite of *Drosophila*. *Evolution* **52**: 832–840.
- Jayakar SD. 1970.** A mathematical model for interaction of gene frequencies in a parasite and its host. *Theoretical Population Biology* **1**: 140–164.
- Jokela J, Dybdahl MF, Lively CM. 1999.** Habitat-specific variation in life-history traits, clonal population structure, and parasitism in a freshwater snail (*Potamopyrgus antipodarum*). *Journal of Evolutionary Biology* **12**: 350–360.
- Jokela J, Lively CM. 1995a.** Parasites, sex and early reproduction in a mixed population of freshwater snails. *Evolution* **49**: 1268–1271.
- Jokela J, Lively CM. 1995b.** Spatial variation in infection by digenetic trematodes in a population of freshwater snails (*Potamopyrgus antipodarum*). *Oecologia* **103**: 509–517.
- Jokela J, Lively CM, Dybdahl MF, Fox JA. 1997a.** Evidence for a cost of sex in the freshwater snail *Potamopyrgus antipodarum*. *Ecology* **78**: 452–460.
- Jokela J, Lively CM, Fox JA, Dybdahl MF. 1997b.** Flat reaction norms and 'frozen' phenotypic variation in clonal snails (*Potamopyrgus antipodarum*). *Evolution* **51**: 1120–1129.
- Keightley PD. 1996.** Nature of deleterious mutation load in *Drosophila*. *Genetics* **144**: 1993–1999.
- Keightley PD, Eyre-Walker A. 2000.** Deleterious mutation and the evolution of sex. *Science* **290**: 331–333.
- Kondrashov AS. 1988.** Deleterious mutations and the evolution of sexual reproduction. *Nature* **336**: 435–440.
- Kondrashov AS. 1993.** Classification of hypotheses on the advantages of amphimixis. *Journal of Heredity* **84**: 372–387.
- Kondrashov AS. 1995.** Modifiers of mutation-selection balance: general approach and the evolution of mutation rates. *Genetical Research* **66**: 53–69.
- Krist AC, Lively CM. 1998.** Experimental exposure of juvenile snails (*Potamopyrgus antipodarum*) to infection by trematode larvae (*Microphallus* sp.): infectivity, fecundity compensation and growth. *Oecologia* **116**: 575–582.
- Krist AC, Lively CM, Levri EP, Jokela J. 2000.** Spatial variation in susceptibility to infection in a snail-trematode interaction. *Parasitology* **121**: 395–401.
- Leslie JF, Vrijenhoek RC. 1980.** Consideration of Muller's Ratchet mechanism through studies of genetic linkage and genomic compatibilities in clonally reproducing *Poeciliopsis*. *Evolution* **34**: 1105–1115.
- Levene H. 1953.** Genetic equilibrium when more than one ecological niche is available. *American Naturalist* **87**: 331–333.
- Little TJ, Ebert D. 2000.** The cause of parasitic infection in natural populations of *Daphnia* (Crustacea: Cladocera): the role of host genetics. *Proceedings of the Royal Society Series B, Biological Sciences* **267**: 2037–2042.
- Little TJ, Ebert D. 2001.** Temporal patterns of genetic variation for resistance and infectivity in a *Daphnia*-microparasite system. *Evolution* **55**: 1146–1152.
- Lively CM. 1986.** Canalization versus developmental conversion in a spatially variable environment. *American Naturalist* **128**: 561–572.
- Lively CM. 1987.** Evidence from a New Zealand snail for the maintenance of sex by parasitism. *Nature* **328**: 519–521.
- Lively CM. 1989.** Adaptation by parasitic trematode to local populations of its snail host. *Evolution* **43**: 1663–1671.
- Lively CM. 1992.** Parthenogenesis in a freshwater snail: reproductive assurance versus parasitic release. *Evolution* **46**: 907–913.
- Lively CM. 1996.** Host-parasite coevolution and sex – do interactions between biological enemies maintain genetic variation and cross-fertilization? *Bioscience* **46**: 107–114.

- Lively CM. 1999.** Migration, virulence, and the geographic mosaic of adaptation by parasites. *American Naturalist* **153**: S34–S47.
- Lively CM. 2001.** Trematode infection and the distribution and dynamics of parthenogenetic snail populations. *Parasitology* **123**: S19–S26.
- Lively CM, Craddock C, Vrijenhoek RC. 1990.** Red Queen hypothesis supported by parasitism in sexual and clonal fish. *Nature* **344**: 864–866.
- Lively CM, Dybdahl MF. 2000.** Parasite adaptation to locally common host genotypes. *Nature* **405**: 679–681.
- Lively CM, Howard RS. 1994.** Selection by parasites for clonal diversity and mixed mating. *Philosophical Transactions of the Royal Society of London B* **346**: 271–281.
- Lively CM, Jokela J. 2002.** Temporal and spatial distributions of parasites and sex in a freshwater snail. *Evolutionary Ecology Research* **4**: 219–226.
- Lively CM, Lyons EJ, Peters AD, Jokela J. 1998.** Environmental stress and the maintenance of sex in a freshwater snail. *Evolution* **52**: 1482–1486.
- Lively CM, McKenzie JC. 1991.** Experimental infection of a freshwater snail, *Potamopyrgus antipodarum*, with a digenetic trematode, *Microphallus* sp. *New Zealand Natural Sciences* **18**: 59–62.
- Lynch M. 1984.** Destabilizing hybridization, general purpose genotypes and geographic parthenogenesis. *Quarterly Review of Biology* **59**: 257–290.
- Lynch M, Conery J, Burger R. 1995a.** Mutation accumulation and the extinction of small populations. *American Naturalist* **146**: 489–518.
- Lynch M, Conery J, Burger R. 1995b.** Mutational meltdowns in sexual populations. *Evolution* **49**: 1067–1080.
- Lynch M, Spitze K, Crease T. 1989.** The distribution of life-history variation in the *Daphnia pulex* complex. *Evolution* **43**: 1724–1736.
- Maynard Smith J. 1978.** *The evolution of sex*. Cambridge: Cambridge University Press.
- Maynard Smith J, Hoekstra RF. 1980.** Polymorphism in a varied environment: How robust are the models? *Genetical Research* **35**: 46–57.
- McFarlane WV. 1945.** The life cycle of *Stegodexamene anquilla* N. G., an allocreadiid trematode from New Zealand. *Parasitology* **41**: 1–10.
- Michaels HJ, Bazzaz FA. 1989.** Individual and population responses of sexual and apomictic plants to environmental gradients. *American Naturalist* **134**: 190–207.
- Michalkiewicz M. 1991.** Macrozoobenthos structure in eutrophic Rosnowskie Duze Lake. *Polskie Archiwum Hydrobiologii* **38**: 427–436.
- Moran NA. 1996.** Accelerated evolution and Muller's ratchet in endosymbiotic bacteria. *Proceedings of the National Academy of Sciences, USA* **93**: 2873–2878.
- Myers MJ, Meyer CP, Resh VH. 2000.** Neritid and thiarid gastropods from French Polynesian streams: how reproduction (sexual, parthenogenetic) and dispersal (active, passive) affect population structure. *Freshwater Biology* **44**: 535–545.
- Negovetic S, Jokela J. 2001.** Life-history variation, phenotypic plasticity, and subpopulation structure in a freshwater snail. *Ecology* **82**: 2805–2815.
- Nei M. 1978.** Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583–590.
- Niklasson H, Parker EDJ. 1994.** Fitness variation in an invading parthenogenetic cockroach. *Oikos* **71**: 47–54.
- Paulissen MA, Walker JM, Cordes JE. 1988.** Ecology of syntopic clones of the parthenogenetic whiptail lizard, *Cnemidophorus laredoensis*. *Journal of Herpetology* **22**: 337–342.
- Pertoldi C, Scali V, Loeschcke V. 2001.** Developmental instability in sexually reproducing and parthenogenetic populations of *Bacillus rossius rossius* and *Bacillus rossius redtenbacheri*. *Evolutionary Ecology Research* **3**: 449–463.
- Peters AD, Keightley PD. 2000.** A test for epistasis among induced mutations in *Caenorhabditis elegans*. *Genetics* **156**: 1635–1647.
- Pfennig DW. 1992.** Polyphenism in spadefoot toad tadpoles as a locally adjusted evolutionarily stable strategy. *Evolution* **46**: 1408–1420.
- Ribi G, Arter H. 1986.** Colonization of Lake Zurich (Switzerland) by the prosobranch snail, *Potamopyrgus jenkinsi*, between 1980 and 1984. *Vierteljahrsschrift der Naturforschenden Gesellschaft in Zürich* **131**: 52–57.
- Rice WR. 1994.** Degradation of a nonrecombining chromosome. *Science* **263**: 230–232.
- Richardson BJ, Baverstock BR, Adams M. 1986.** *Allozyme electrophoresis: a handbook for animal systematics and population studies*. San Diego: Academic Press.
- Roff DA. 1994.** Evidence that the magnitude of the trade-off in a dichotomous trait is frequency dependent. *Evolution* **48**: 1650–1656.
- Schlosser IJ, Doeringsfeld MR, Elder JF, Arzayus LF. 1998.** Niche relationships of clonal and sexual fish in a heterogeneous landscape. *Ecology* **79**: 953–968.
- Schreiber ESG, Glaister A, Quinn GP, Lake PS. 1998.** Life history and population dynamics of the exotic snail *Potamopyrgus antipodarum* (Prosobranchia: Hydrobiidae) in Lake Purrumbete, Victoria, Australia. *Marine and Freshwater Research* **49**: 73–78.
- Seger J, Brockmann HJ. 1987.** What is bet-hedging? *Oxford Surveys in Evolutionary Biology* **4**: 182–211.
- Semlitsch RD. 1993.** Adaptive genetic variation in growth and development of tadpoles of the hybridogenetic *Rana esculenta* complex. *Evolution* **47**: 1805–1818.
- Semlitsch RD, Hotz H, Guex GD. 1997.** Competition among tadpoles of coexisting hemiclones of hybridogenetic *Rana esculenta*: support for the Frozen Niche Variation model. *Evolution* **51**: 1249–1261.
- Sinervo B, Lively CM. 1996.** The rock-paper-scissors game and the evolution of alternative male strategies. *Nature* **380**: 240–243.
- Stenberg P, Terhivuo J, Lokki J, Saura A. 2000.** Clone diversity in the polyploid weevil *Otiorhynchus scaber*. *Heredity* **132**: 137–142.
- Stoddard JA. 1983.** A genotypic diversity measure. *Journal of Heredity* **74**: 489–490.
- Stoddard JA, Taylor JF. 1988.** Genotypic diversity: esti-

- mation and prediction in samples. *Genetics* **118**: 705–711.
- Timms BV. 1982.** A study of the benthic communities of 20 lakes in the South Island, New Zealand. *Freshwater Biology* **12**: 123–138.
- Towns DR. 1981.** Life histories of benthic invertebrates in a Kauri Forest stream in northern New Zealand. *Australian Journal of Marine and Freshwater Research* **32**: 191–212.
- de Visser JAGM, Hoekstra RF, Van Den Ende H. 1997.** An experimental test for synergistic epistasis and its application in *Chlamydomonas*. *Genetics* **145**: 815–819.
- Vrijenhoek RC. 1978.** Coexistence of clones in a heterogeneous environment. *Science* **199**: 549–552.
- Vrijenhoek RC. 1979.** Factors affecting clonal diversity and coexistence. *American Zoologist* **19**: 787–797.
- Vrijenhoek RC. 1984.** Ecological differentiation among clones: the frozen niche variation model. In: Levin SA, ed. *Population biology and evolution*. Berlin: Springer-Verlag, 217–231.
- Vrijenhoek RC. 1990.** Genetic diversity and the ecology of asexual populations. In: Wöhrmann K, Jain SK, eds. *Population biology, ecological and evolutionary viewpoints*. Berlin: Springer-Verlag, 175–197.
- Vrijenhoek RC, Lerman S. 1982.** Heterozygosity and developmental stability under sexual and asexual breeding systems. *Evolution* **36**: 768–776.
- Wallace C. 1992.** Parthenogenesis, sex and chromosomes in *Potamopyrgus*. *Journal of Molluscan Studies* **58**: 93–107.
- Ward J, Talbot J. 1984.** Distribution of aquatic macrophytes in Lake Alexandrina, New Zealand. *New Zealand Journal of Marine and Freshwater Research* **18**: 211–220.
- Weeks AR, Hoffmann AA. 1998.** Intense selection of mite clones in a heterogeneous environment. *Evolution* **52**: 1325–1333.
- Weetman D, Hauser L, Carvalho GR. 2001.** Isolation and characterization of di- and trinucleotide microsatellites in the freshwater snail *Potamopyrgus antipodarum*. *Molecular Ecology Notes* **1**: 185.
- Weider LJ. 1993.** A test of the general-purpose genotype hypothesis – differential tolerance to thermal and salinity stress among *Daphnia* clones. *Evolution* **47**: 965–969.
- West SA, Lively CM, Read AF. 1999.** A pluralist approach to sex and recombination. *Journal of Evolutionary Biology* **12**: 1003–1012.
- Wetherington JD, Weeks SC, Kotora KE, Vrijenhoek RC. 1989.** Genotypic and environmental components of variation in growth and reproduction of fish hemiclones (Poeciliopsis: Poeciliidae). *Evolution* **43**: 635–645.
- Williams GC. 1975.** *Sex and evolution*. Princeton: Princeton University Press.
- Winterbourn MJ. 1974.** Larval Trematoda parasitizing the New Zealand species of *Potamopyrgus* (Gastropoda: Hydrobiidae). *Mauri Ora* **2**: 17–30.