Oecologia (2005) 143: 527–536
DOI 10.1007/s00442-005-0016-5

POPULATION ECOLOGY

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Outcomes of reciprocal invasions between genetically diverse and genetically uniform populations of *Daphnia obtusa* (Kurz)

Received: 6 December 2004 / Accepted: 19 January 2005 / Published online: 14 April 2005

Abstract Ecological theory predicts that genetic variation produced by sexual reproduction results in niche diversification and provides a competitive advantage both to facilitate invasion into genetically uniform asexual populations and to withstand invasion by asexual competitors. We tested the hypothesis that a large group of diverse clones of *Daphnia obtusa* has greater competitive advantage when invading into genetically uniform populations of this species than a smaller group with inherently less genetic diversity. We compared competitive outcomes to those of genetically uniform groups of small and large size invading into genetically diverse populations. Genetically diverse invaders of initially large group size increased their representation by more than those of initially small size; in contrast, genetically uniform invaders of initially large group size diminished on average by more than those of initially small size. These results demonstrate an advantage to the genetic variation produced by sexual reproduction, both in invasion and resisting invasion, which we attribute to competitive release experienced by individuals in genetically diverse populations.

Keywords Cost of males · Cost of sex · Density dependence · Niche breadth

Communicated by Roland Brandl

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Introduction

Understanding why sexual reproduction prevails in the natural world over faster reproducing asexual modes is held as one of the great challenges of evolutionary biology (e.g. West et al. 1999), yet the core issue of prevalence requires a purely ecological analysis of competition between reproductively isolated sexual and asexual species (Doncaster et al. 2000). Sexual reproduction has an immediate cost relative to asexual reproduction because of its investment in male gametes that can only contribute to population growth through female gametes. This is the widely accepted twofold cost of sex for anisogamous species in which half the investment in reproduction comprises males or male gametes (Williams 1975). An asexual mutant arising in such a sexual population will double its relative representation in the recipient population in successive generations by producing only fecund females, all else being equal (Maynard-Smith 1978). Mutant parthenogens do arise amongst sexual populations, even of some vertebrates (e.g. lizards: Case 1990; geckos: Radtkey et al. 1995). The paradox of sex is that the evolutionary advantage to sex of its genetic variability appears sufficient to counter this immediate ecological cost for many species. Although genetic variation can reduce sib-competition (Bell 1982), empirical tests have shown a consistently less than twofold drop in sib-competition within a sexual population compared to the sib-competition within an asexual population (e.g. Schmitt and Ehrardt 1987; Kelley 1989). Recent explanations for the maintenance of sex have favoured a synergy of evolutionary processes (e.g. West et al. 1999), involving mutation accumulation (Muller's ratchet) and frequency-dependent selection of host genotypes driven by parasites (Red Queen model of Hamilton et al. 1990).

All such models seek a twofold advantage to genetic variation that might act sufficiently quickly to counter the immediate cost of males. Yet, classical ecological

theory predicts a much less than twofold cost of males in crowded environments. The ecological models of Case and Taper (1986), Gaggiotti (1994), Doncaster et al. (2000, 2003), Kerszberg (2000), Pound et al. (2002, 2004) all show how male presence can impose a considerably less than twofold cost on population growth for sexual populations at density-dependent carrying capacity. For example, the Lotka-Volterra model of Doncaster et al. (2000) demonstrates that a sexual population requires only a small average *per capita* advantage in competition with an invading asexual mutant to halt the invasion process. It suffices for the asexual competitors to exert a slightly weaker inhibitory effect on the carrying capacity of the sexual population than they exert on their own carrying capacity through intra-specific competition. The resulting competitive release for the sexual population permits coexistence with the asexual invader on a depleted resource base both locally and regionally (Doncaster et al. 2003). Under these crowded conditions, the sexual population may eventually drive out the asexual competitor by virtue of the longer-term benefits of sexual recombination (e.g. Kondrashov 1993; Dunbrack et al. 1995; Pound et al. 2004). The small competitive release can suffice even to resist multiple clonal invasions (detailed in Pound et al. 2002, responding to West and Peters 2000). Although these predictions concern population-level outcomes, their derivation from analyses of *per capita* fitness does not require any group selection argument (contrary to the claim of Getz 2001).

This class of models for competitive release has yet to be tested experimentally, despite a growing body of empirical evidence in favour of a substantial ecological component to the cost of sex. Asexual clones arising from a sexual population may each contain only a small sample of the genetic variation within the parent population (Vrijenhoek 1979; Hebert et al. 1988; Honeycutt and Wilkinson 1989; Semlitsch et al. 1997). The resulting narrower niche for the clones may favour coexistence at local or regional scales (Case 1990; Barata et al. 1996; Fox et al. 1996; Vrijenhoek and Pfeiler 1997; Negovetic et al. 2001).

Tagg et al. (2005) have directly tested the ecological model of Doncaster et al. (2000) by measuring competitive impacts between different clonal assemblies of *Daphnia pulex*. A genetically varied population of females had significantly lower birth rates in competition with itself than in competition with genetically uniform populations of females cultured from the same stock, demonstrating competitive release. The Lotka-Volterra coefficient for this interaction was estimated at: $\alpha_{12} = 0.896$, indicating a relatively small competitive advantage afforded by genetic diversity. Its value was nevertheless sufficiently below unity to predict persistence for a genetically varied population having half the growth capacity of the genetically uniform competitor, which is the expectation for a sexual population investing equally in production of males and females, competing with an asexual sibling species.

These theoretical and empirical studies of the ecological costs of sex give rise to a new hypothesis that we test in this paper. If genetic variation confers competitive advantage, a large group of genetically diverse individuals should have a greater advantage than a small group, with inherently less genetic diversity. We explored the effects on a genetically uniform recipient population of invasion by a small or a large clonal assemblage of *Daphnia obtusa* Kurz (Crustacea: Cladocera). We also explored the reciprocal effects on a genetically diverse recipient population of invasion by a small or large single-clone group. We tested two hypotheses: (1) a genetically diverse group will invade into a genetically uniform recipient population, possibly replacing it, whereas a genetically uniform group will decrease within a genetically diverse recipient population, possibly being eliminated from it; and (2) these effects will be more pronounced for large than for small invading groups.

The experiment used cyclically parthenogenetic *D. obtusa* during parthenogenetic growth. The aquatic habitat and fast growth of *Daphnia* facilitate measuring competitive impacts, and its parthenogenetic (asexual) phase allows control over genetic diversity without the confounding effects of the presence of males or the cost of searching for a mate and mating. We were therefore able to test for competitive advantages due to the genetic variation produced by sexual reproduction, but in isolation from the growth capacity disadvantage of producing males.

All-female populations of free-living *D. obtusa* reproduce asexually until unfavourable conditions arise (for example over-crowding, reduced food or a change in temperature) when males are produced parthenogenetically. Sexual females then produce haploid eggs that are fertilized by these males, resulting in resting eggs protected by the ephippium (modified carapace) that are able to withstand extreme conditions of freezing or desiccation. The resting eggs hatch upon resumption of favourable conditions, enabling *Daphnia* populations to persist in temporary habitats. Some species of *Daphnia* have abandoned the sexual phase of their life cycle in favour of obligate parthenogenesis. Populations of obligately parthenogenetic *D. pulex* occur in Canada (Hebert et al. 1988) and further south (Lynch et al. 1989). Obligate parthenogens produce diapausing eggs that do not require fertilisation and will hatch into genetically identical offspring. The origin of new obligate parthenogenetic clones has been suggested to result from the males produced by some obligate parthenogens transmitting a dominant gene for sex-limited meiosis suppression when mating with sexual females from cyclical parthenogenetic *D. pulex* (Innes and Hebert 1988). Evidence that these males can pass such genes to their progeny suggests that the gene could spread through a cyclically parthenogenetic population, resulting in a genotypically diverse group of obligate parthenogens. It is reasonable to assume that this process is continuing and therefore that some obligate partheno-

gen clones have originated relatively recently (Innes and Hebert 1988). The arrival of such obligate parthenogen mutants into a genetically diverse population perfectly demonstrates a natural invasion of the sort replicated by this experiment in the laboratory.

Materials and methods

Daphnia cultures

Laboratory cultures of *D. obtusa* clones were derived from single post-hatching females taken from Pig Bush Pond in the New Forest, Hampshire, UK, immediately after the pond had refilled in late October (the favourable season for *Daphnia* being October–May). Cellulose acetate electrophoresis was performed on a random sample of *Daphnia* (Hebert and Beaton 1989) for the allozyme glucose-6-phosphate isomerase (GPI). This confirmed the population to be in Hardy-Weinberg equilibrium (SS = 203, SF = 190, FF = 63; $\chi^2 = 2.975$, df = 1, $P > 0.05$). Each clonal lineage was established from a healthy female of large size, carrying a brood. Following acclimatisation, electrophoresis was performed on one female from each monoclonal culture, to determine its GPI genotype. All cultures displaying FF homozygosity were discarded, whilst those displaying SS homozygosity or SF heterozygosity were retained, until 177 healthy cultures had been distinguished of each of these two genotypes. Since the seed females were taken from a population recently established from sexual eggs, it is assumed that clones with the same GPI genotype were genetically different for other loci as has been found for other cyclically parthenogenetic populations of *Daphnia* in temporary ponds (Young 1979; Hebert et al. 1988). Each of the 354 cultures was maintained in a plastic, open-mouthed container, holding approximately 1.5 l zooplankton media (Lynch et al. 1986), and fed ad libitum on aquaria-cultured algae, containing bacteria and inedible algae as well as edible algae. The cultures were kept in a temperature-controlled room set at 19°C for a 16L:8D photoperiod, until they became healthy populations of at least 100 individuals. In addition, a random selection of four SS and four SF clones were cultured to population sizes approaching 1,000 individuals.

Experimental design

A total of 32 experimental treatments were paired into two conditions: the invasion of genetically diverse groups into genetically uniform populations, and the reciprocal invasion of genetically uniform groups into genetically diverse populations. Populations and invading groups were assembled by picking non-brood-carrying females from cultures, approximately 1 week prior to reproductive maturity. Each invasion event was repeated for groups of small and large size (10 and 100 individuals, respectively) invading into the recipient population of 200 individuals, and for GPI genotype status (SF/SS or SS/SF for invader/recipient). Figure 1 shows how the genotype functioned as a marker to distinguish between the genetically diverse and genetically uniform populations in each treatment.

Each genetically uniform recipient population was created by taking 200 females from one of the large cultures. Two containers were prepared for each of eight such recipient populations, using the four different cultures of genotype SF and the four of genotype SS. The switching of the GPI marker allowed control over any differences in life-history traits between genotypes. Genetically diverse invading groups were assembled by taking one female from each of ten randomly chosen cultures to create the smaller invading group, and one female from each of 100 cultures to create the larger invading group. Eight genetically diverse invading groups were thus prepared, four of genotype SF and four of genotype SS, and each represented by a large and a small group size.

Similarly, each genetically diverse recipient population was assembled by taking one female from each of the 177 cultures of the same genotype status, plus another female from each of 23 of the cultures chosen at random to make the numbers up to 200. Two containers were prepared for each of the four recipient populations of genotype SF and four of genotype SS. Each genetically uniform invading group was created from either 10 or 100 individuals of the same clone, chosen from the large cultures (as for the genetically uniform recipient populations). Eight genetically uniform invading groups were thus prepared, four of genotype SF and four of genotype SS, and each represented by a large and a small group size.

Invasion events were initiated simultaneously for small and large group sizes. For each invading group size, the four genetically diverse groups of genotype SF were injected into the four genetically uniform populations of genotype SS and vice versa, and the four

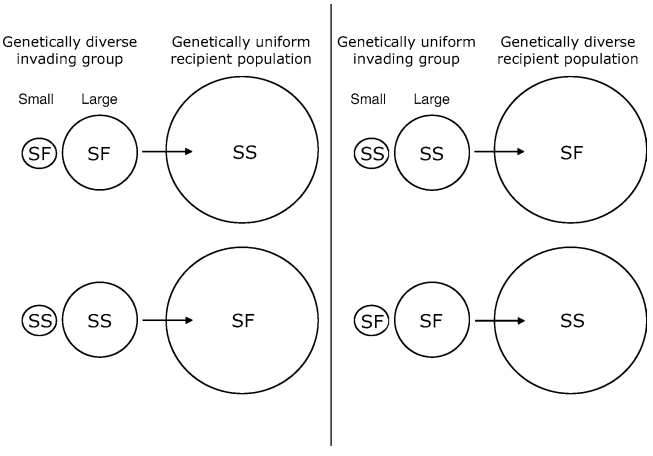

Fig. 1 Experimental design. Small and large genetically diverse and genetically uniform invading groups were injected into genetically uniform and genetically diverse recipient populations, respectively

genetically diverse groups of genotype SS were injected into the four genetically uniform populations of genotype SF and vice versa.

Feeding and monitoring

Each recipient population was housed in 1.4 l zooplankton media (7 ml per individual *Daphnia*) in a plastic open-mouthed, 2.5-l container, to which the appropriate invading group was added. The volume of media reflected the total recipient population at the start of the experiment, and was maintained at this level regardless of invading group size or any fluctuations in population size during the experiment. Zooplankton media were topped up when necessary and all populations had their media changed when required. Any settled inedible algae, detritus or dead *Daphnia* was removed daily. Treatments were fed three to five times a week with $2.0–2.4\times10^6$ algal cells, or 100,000–120,000 cells per *Daphnia* (haemocytometer counts), grown in an aquarium housing a goldfish. Previous experiments have established that this is less than ad libitum and therefore provided conditions of food stress (Tagg et al. 2005). Such aquaria-cultured algae water contains inedible algae and bacteria as well as edible bacteria, providing a multi-dimensional niche conducive to clone-specific responses. An impure food supply can also dampen booms and crashes in the population if inedible algae utilise the nutrients required by the edible algae (McCauley et al. 1999). All treatments were housed in a temperature-controlled room at 19°C for a 16L:8D h photoperiod.

Estimations of invader proportion and population size

Data were collected after 3, 6, 9 and 13 weeks, to encompass establishment and growth phases of the invasions. Cellulose acetate electrophoresis for the allozyme GPI was carried out on 48 randomly selected individuals (two applications of 24 individuals per plate). This resulted in two gels being run per treatment and 64 gels in total for each analysis. Individuals in the sample were classed as GPI genotype SS or SF and the proportion of the invader genotype in the sample was recorded. Population counts were made from 100-ml samples extracted from the agitated media, and multiplied by 14 to estimate the total number of *Daphnia* present in the 1.4-l container. *Daphnia* were returned to their containers after the count.

Statistical analysis

A generalised linear model design (glm in S-Plus version 6.2) was used to test the null hypothesis that the proportion of the population comprising invaders was independent of the initial size of the invading group (10 or 100), mode of invader (genetically diverse or genetically uniform) and genotype of invader (SF or SS). Table 1 shows the balanced model with four replicate random clones nested in each of two levels of genotype and cross-factored with two levels of mode and two of size. The hypothesised effects of genetic diversity were sought in the interaction of size × mode. This repeated measures design makes the inherent assumption of no significant interaction of size × mode with clone (nested in genotype), which cannot be tested directly because it has no estimable residual error.

The proportional responses were modelled with binomial error structure and logit link, correcting for over-dispersion by applying *F*-tests to the analysis of deviance table (Crawley 2002). The treatment variance for genotype was tested against the error variance for clone (nested in genotype), and the treatment variance for each of the other factors and their interactions was tested against the error variance of its interaction with the clone (nested in genotype). The binomial error structure causes deviances to depend on their order of entry into the model. The model was therefore run four times, switching the entry order of effects and recording the deviances only of the last entered effect, in order to adjust each deviance for all terms at the same level or lower in the model hierarchy. The analysis was repeated at 3, 6, 9 and 13 weeks in preference to factoring time into a single analysis because variances increased with time.

Population sizes were transformed by raising counts to the power of 0.45, and subjected to a single ANOVA with Gaussian errors using the same model as Table 1 but including the extra cross-factor of time (with four levels: 3, 6, 9, 13 weeks).

Table 1 The glm design S|C′(G)|M used for analysis at each stage in the study

S|C′(G)|M	$M_{diverse}$								$M_{uniform}$							
	G_{SF}				G_{SS}				G_{SF}				G_{SS}			
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8
S_{10}	O_1	O_3	O_5	O_7	O_9	O_{11}	O_{13}	O_{15}	O_{17}	O_{19}	O_{21}	O_{23}	O_{25}	O_{27}	O_{29}	O_{31}
S_{100}	O_2	O_4	O_6	O_8	O_{10}	O_{12}	O_{14}	O_{16}	O_{18}	O_{20}	O_{22}	O_{24}	O_{26}	O_{28}	O_{30}	O_{32}

S size, *G* genotype, *M* mode of reproduction of invading group, *C* random clone of the genetically uniform component, *O* observation of proportionate size of invading group (out of 48 individuals)

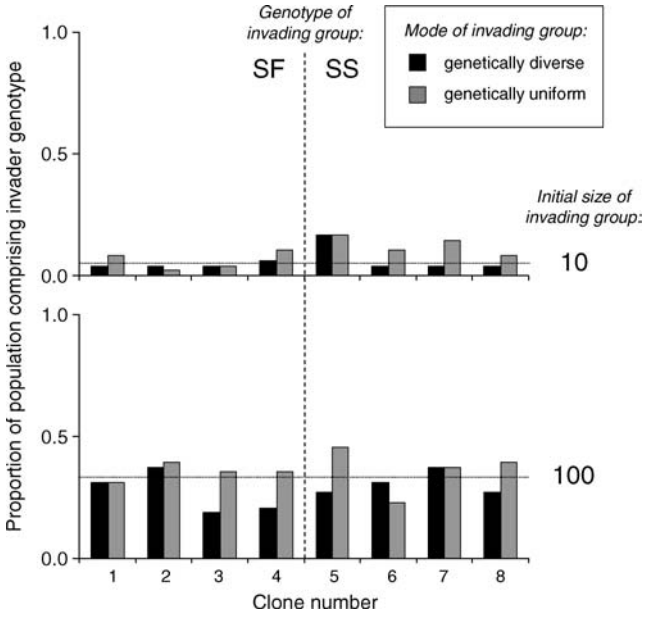

Fig. 2 Proportion of the population comprising the invader genotype after 3 weeks in the recipient population, depending on initial size of invading group (10 = top graph, 100 = bottom) cross-factored with mode of invader (genetically diverse *black bars*, uniform *grey bars*), and with clone of uniform component (*x*-axis) nested in Genotype of invader (SF Clones 1–4, SS clones 5–8). *Horizontal dotted lines* give the initial invader proportions

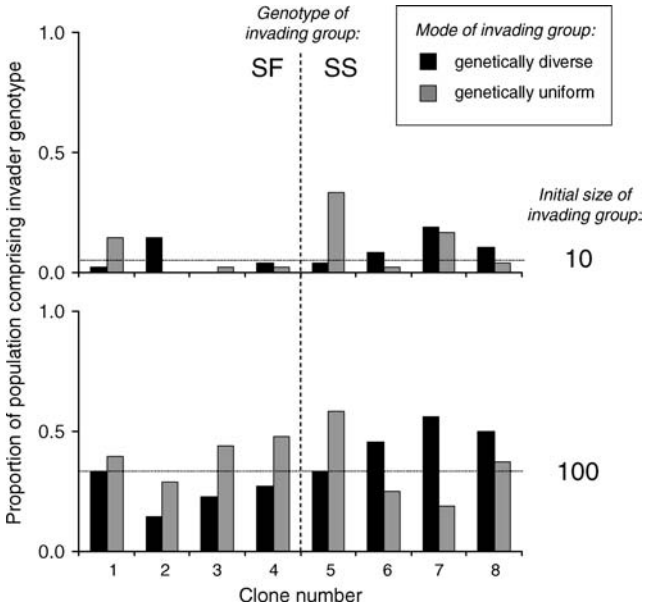

Fig. 3 Invader response after 6 weeks (format as Fig. 2)

Results

Invader proportions over time

At 3 weeks, Fig. 2 shows populations reflecting their initial invader proportions, given by the dotted horizontal lines (Table 2, size $F_{1,6} = 120.42$, $P < 0.01$). No differences were apparent by genotype or mode at this stage, and there were no interacting effects.

At 6 weeks, Fig. 3 shows the populations generally still reflecting their initial invader proportions (Table 2, size $F_{1,6} = 65.33$, $P < 0.01$), and the invader genotype SS generally outperforming SF (genotype $F_{1,6} = 6.75$, $P < 0.05$). Invader proportions did not depend on mode and there were no interacting effects.

At 9 weeks, Fig. 4 shows the populations still reflecting their initial invader proportions, albeit with a weaker size effect (Table 2, size $F_{1,6} = 11.62$, $P < 0.05$), and two initially small groups of genetically uniform invaders having outgrown their initially large-group counterparts (clones 5 and 7). As previously, invader genotype SS was generally outperforming SF (genotype $F_{1,6} = 10.06$, $P < 0.05$). Invader proportions did not depend on mode and there were no interacting effects.

At 13 weeks, Fig. 5 shows the strongest invaders being the genetically diverse groups of initially large size, all but one of which maintained or increased in proportion in contrast to the genetically uniform invaders of initially large size which all reduced in proportion

Table 2 Results of the glm with binomial error structure and a logit link function

Source	df	At 3 weeks		At 6 weeks		At 9 weeks		At 13 weeks	
		Variance	*F*	Variance	*F*	Variance	*F*	Variance	*F*
G	1	3.06	0.37	15.19	6.75*	52.51	10.06*	108.67	3.85
M	1	7.22	1.67	0.83	0.14	21.98	1.37	206.01	49.40**
S	1	156.55	120.42**	182.93	65.33**	92.92	11.62*	45.65	4.54
C(G)	6	8.28	–	2.25	–	5.22	–	28.25	–
G×M	1	0.02	0.00	9.83	1.63	2.71	0.17	0.04	0.01
G×S	1	2.75	2.12	4.53	1.62	1.15	0.14	0.24	0.02
M×S	1	0.28	0.24	0.73	0.20	14.71	5.11	23.97	5.59***
G×M×S	1	0.57	0.50	4.52	1.23	7.99	2.77	12.54	2.92
M×C(G)	6	4.32	–	6.03	–	16.00	–	4.17	–
S×C(G)	6	1.30	–	2.80	–	8.00	–	10.06	–
Error	6	1.15		3.66		2.88		4.29	
Total	31	6.34		9.94		11.61		21.46	

Symbols as for Table 1; variance = deviance/df
$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P = 0.055$

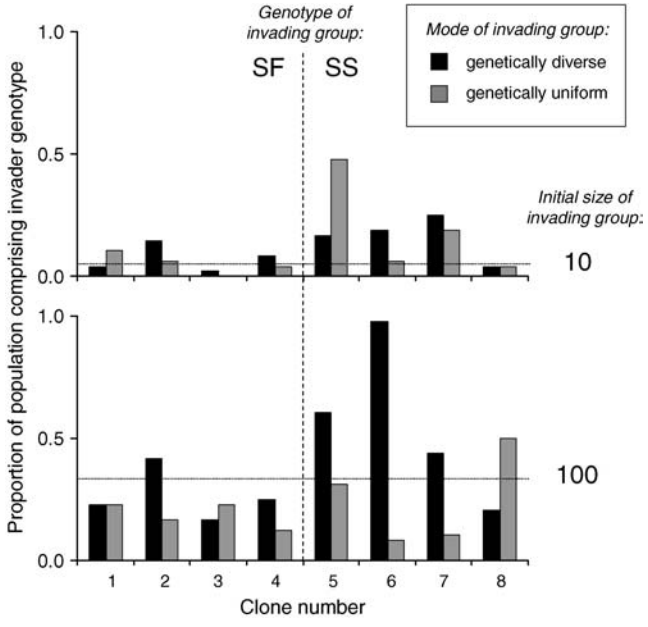

Fig. 4 Invader response after 9 weeks (format as Figs. 2, 3)

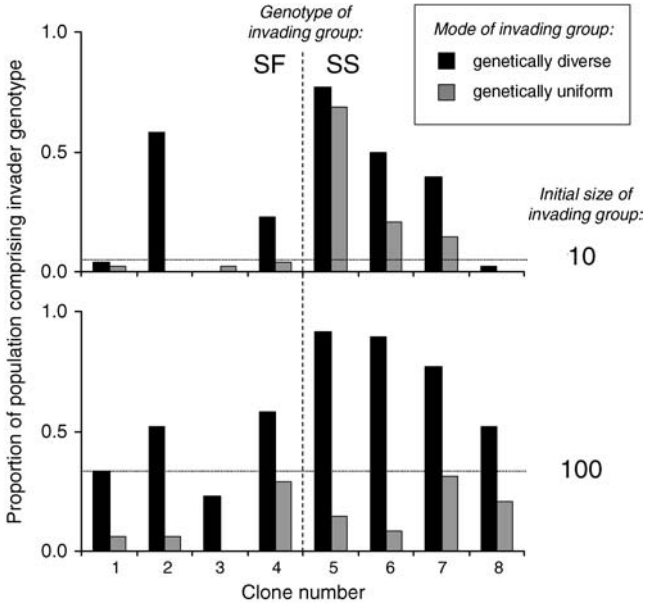

Fig. 5 Invader response after 13 weeks (format as Figs. 2, 3 and 4)

(Table 2, marginal significance of size × mode $F_{1,6} = 5.59$, $P = 0.055$). The genetically diverse invading groups generally outperformed the genetically uniform invading groups (mode $F_{1,6} = 49.40$, $P < 0.01$), to the extent that there was no longer a discernible main effect of initial group size (size $F_{1,6} = 4.54$, $P = 0.08$). The variation within genotypes increased more than the variation between them (genotype $F_{1,6} = 3.85$, $P = 0.10$, and no significant interactions with genotype).

The repeated-measures assumption of no interaction of size × mode with clone (nested in genotype) can be evaluated qualitatively at week 13. Perusal of Fig. 5 indicates a bigger difference between the black and grey bars in the lower graph than the upper graph for almost all clones, indicating a generally consistent response between clones within each level of size × mode. This is particularly marked amongst the invaders of genotype SS, and the two exceptions arise in the SF invaders (clones 2 and 3).

Rise and fall of invading groups

At 13 weeks, almost half of the invading groups had risen in proportion relative to their starting ratios (14 out of 32, Fig. 5). Eleven of these were genetically diverse invading groups, and three were genetically uniform invading groups (Fisher exact test: $P = 0.004$). No invading groups entirely ousted their recipient population. Three genetically diverse recipient populations extirpated their genetically uniform invading groups (clones 2 and 8 of initially small size and clone 3 of initially large size); one genetically uniform recipient population (clone 3) extirpated its genetically diverse invading group (of initially small size).

Amongst the genetically diverse invading groups, the majority that maintained or increased their size were of initially large size (7 of 12), and the majority that decreased in size were of initially small size (3 of 4). Amongst the genetically uniform invading groups in contrast, all three that increased in size were of initially small size, and the majority that decreased were of initially large size (8 of 13).

Of the eight clones representing the genetically uniform populations as either invader or recipient, each decreased in frequency from its initial starting proportion in at least three of its four trials (Table 3, decreases in 26 of 32 trials). The most marked exception was a significant increase in frequency by clone 5 when acting as a small invading group into a genetically diverse recipient population, contrasting with its significant decrease for the other treatments.

Population sizes

Figure 6 shows the populations fluctuating during the course of the experiment (time $F_{3,18} = 52.09$, $P < 0.001$) without any remarkable crashes or booms, but influenced by the initial size of the invading group (size × time $F_{3,18} = 28.04$, $P < 0.001$). Population size did not depend on other main effects or interactions.

Discussion

The events of genetically uniform groups invading genetically diverse populations relate to the ecological pressure on anisogamous sexual populations wherever asexual mutants arise. Asexual mutants gain an intrinsic

Table 3 Changes over time in the proportional representation of each of eight clones according to its starting proportion. Significance of change is based on a *G*-test with four df, followed by a sequential Bonferroni correction ($^{*}P < 0.05$)

Clone	Population	GPI	Starting proportion	Clone proportion in week				Direction of change
				3	6	9	13	
1	Invading	SF	0.048	0.083	0.146	0.104	0.021	−
1	Invading	SF	0.333	0.313	0.396	0.229	0.063	−*
1	Recipient	SF	0.952	0.958	0.979	0.958	0.958	.
1	Recipient	SF	0.667	0.688	0.667	0.771	0.667	−
2	Invading	SF	0.048	0.021	0.000	0.063	0.000	−
2	Invading	SF	0.333	0.396	0.292	0.167	0.063	−*
2	Recipient	SF	0.952	0.958	0.854	0.854	0.417	−*
2	Recipient	SF	0.667	0.625	0.854	0.583	0.479	−*
3	Invading	SF	0.048	0.042	0.021	0.000	0.021	−
3	Invading	SF	0.333	0.354	0.438	0.229	0.000	−*
3	Recipient	SF	0.952	0.958	1.000	0.979	1.000	+
3	Recipient	SF	0.667	0.813	0.771	0.833	0.771	−
4	Invading	SF	0.048	0.104	0.021	0.042	0.042	−
4	Invading	SF	0.333	0.354	0.479	0.125	0.292	−
4	Recipient	SF	0.952	0.938	0.958	0.917	0.771	−
4	Recipient	SF	0.667	0.792	0.729	0.750	0.417	−*
5	Invading	SS	0.048	0.167	0.333	0.479	0.688	+*
5	Invading	SS	0.333	0.458	0.583	0.313	0.146	−*
5	Recipient	SS	0.952	0.833	0.958	0.833	0.229	−*
5	Recipient	SS	0.667	0.729	0.667	0.396	0.083	−*
6	Invading	SS	0.048	0.104	0.021	0.063	0.208	+
6	Invading	SS	0.333	0.229	0.250	0.083	0.083	−*
6	Recipient	SS	0.952	0.958	0.917	0.813	0.500	−*
6	Recipient	SS	0.667	0.688	0.542	0.021	0.104	−*
7	Invading	SS	0.048	0.146	0.167	0.188	0.146	.
7	Invading	SS	0.333	0.375	0.188	0.104	0.313	−/+*
7	Recipient	SS	0.952	0.958	0.813	0.750	0.604	−*
7	Recipient	SS	0.667	0.625	0.438	0.563	0.229	−*
8	Invading	SS	0.048	0.083	0.042	0.042	0.000	−
8	Invading	SS	0.333	0.396	0.375	0.500	0.208	−
8	Recipient	SS	0.952	0.958	0.896	0.958	0.979	+
8	Recipient	SS	0.667	0.729	0.500	0.792	0.479	−*

advantage by avoiding the cost of investing in males or male gametes. Males were not present in any of our experimental populations, although they arise in natural populations of sexually reproducing *D. obtusa* as environmental conditions begin to deteriorate. Any advantage to genetic diversity in natural populations of *Daphnia* of the sort measured by this experiment will be counterbalanced to some degree by the intrinsic cost to growth capacity of producing males that sustain the genetic diversity. Since the design did not impose any carrying capacity on populations, we were not able to estimate competition coefficients and thereby to quantify the trade-off between competitive release and growth capacity that would be experienced by sexual and asexual populations in competition with each other (cf. Tagg et al. 2005). It is beyond the scope of this experiment to know whether the responses of invading groups were due to higher realised fecundity of the better competitor or greater realised mortality of the worse competitor.

Despite these design limitations, we have been able to show that genetically diverse populations provided more resistance to invasion by a genetically uniform invader than genetically uniform populations could provide to a genetically diverse group of invaders. Furthermore, the large genetically diverse groups invading into genetically uniform recipient populations tended to outperform both small genetically diverse groups and genetically uniform groups invading into genetically diverse populations. It may appear as a foregone conclusion that a population of clones will do better than a single clone from the same population, yet precisely this result is needed to demonstrate an ecological (competitive) advantage to genetic variation, by which sexual reproduction can compensate for its evolutionary (intrinsic) cost in growth capacity due to the presence of males. To our knowledge, these are the first empirical tests of the contributions of genetic variation to interspecific competition, in contrast to previous tests which focused on intraspecific (sib-) competition (e.g. Ellstrand and Antonovics 1985; Schmitt and Ehrardt 1987; Kelley 1989; Bell 1990). Weeks (1995) tested fitness indicators of pseudogamous clones and out-crossed sexual strains of the fish *Poeciliopsis*, finding that sexual populations outperformed clones when held in isolation, but not under competition in mixed treatments. Recent ecological models of pseudogamy suggest that sperm-dependent parthenogenesis imposes a special set of selection pressures, whereby sperm-dependency can compensate for lower intrinsic growth with a superior intrinsic resource exploitation (Schley et al. 2004).

In our previous experiments (Tagg et al. 2005), we recorded a slight but significant competitive release for a small population of genetically varied *Daphnia* in exploitation competition with replicate populations of genetically uniform individuals ($\alpha_{12} \approx 0.9$). This advantage was attributable explicitly to clone-specific responses to the multi-dimensional niche provided by aquaria-cultured algae water. Both the previous and current experiments sustained populations with a limited supply of the impure food mix. We therefore expect that the genetically uniform populations in the current experiment likewise suffered from a narrower feeding niche, enabling the large genetically diverse groups in particular to increase their relative representation. We cannot discount the possibility from this experiment, however, that successful groups were expressing an advantage solely in intrinsic rates of increase, for example due to fewer deleterious mutations, rather than in competitive release due to niche breadth. Against this possibility, the eight clones that could be tracked in the experiments provided no evidence for large differences between them in population growth (Table 3); similarly Tagg et al. (2005) found no difference between clones in intrinsic birth rates. Such intrinsic advantages as may have existed for some clones will have acted to the disadvantage of competing clones regardless of whether they belonged to the genetically diverse or uniform group or population. We therefore expect intrinsic differences to contribute to noise in the results, rather than determining the 13-week outcome of genetically diverse groups or populations increasing in 26 of the 32 trials (Fig. 5 and Table 3).

The dominance of the genetically diverse population after 13 weeks raises the question of whether this was achieved by a small or a large subset of the original invading clones. Although we could not follow the success of individual genotypes making up the genetically diverse population, we have noted the absence of systematic differences between the eight clones used to produce the genetically uniform groups and populations (Table 3). Combined with the small number of known extinctions (three clones of genetically uniform groups and ten clones of one small genetically diverse group), this suggests a predominating coexistence at 13 weeks rather than exclusion of all but the most successful clone (again consistent with niche differences rather than intrinsic differences). Our expectation that most clones starting these experiments will have persisted is supported by other laboratory competition experiments showing changes in the frequency of different *Daphnia* clones during similar timescales (Weider 1992; Innes and Singleton 2000; Capaul and Ebert 2003; Haag and Ebert 2004). For example, Haag and Ebert (2004) found only small changes in *Daphnia magna* clonal diversity and all clones persisted in an experiment involving 19 different clones over a 4-month period. Recent studies have also found that clonal variation in susceptibility to parasites can affect the outcome of competition among *Daphnia* clones (Capaul and Ebert 2003; Haag and Ebert 2004). Sufficient controls were used in the present experiments such that any differential effects of parasites would have been detected as changes in clone frequency (Table 3).

The experiment recorded an absolute disadvantage for large compared to small genetically uniform invading groups, in addition to the disadvantage relative to the genetically diverse invading groups (Fig. 5, each grey bar lies below the dotted line in the lower graph as well as being below its black-bar pair). The absolute reductions in size from large starting proportions reflect the fact that large groups had more individuals to lose than small groups. Likewise, the absolute expansions of large genetically diverse invading groups (Fig. 5, all but one black bar lies at or above the dotted line in the lower graph) reflect a long-term advantage that followed earlier losses (cf. Figs. 2, 3 and 4). The relative and absolute gains for large genetically diverse populations accrued over a period of more than 3 months, and Figs. 2, 3, 4 and 5 show the emerging pattern.

Since the experiment began with all invading groups and recipient populations comprising young non-brood-carrying female *Daphnia*, they required the first week to mature and begin to produce young. Food supply was limited to maintain approximately the same number of *Daphnia* in the original population, so many of the young produced may not have survived into adulthood before the original adults began to age and die. The fluctuations in population sizes during the course of the experiment (Fig. 6) can be partly explained by these dynamics. Once reproductively mature, *Daphnia* can produce as many as 30 genetically identical young every few days for their 40-day lifespan. Generation times are therefore short and population growth rapid, but the effects of population turnover were not expected to become visible until about the third month of the experiment.

The potential for sexual reproduction to balance its cost of males in competitive release depends on the particular traits of competing asexual clones. Our experiment used four clonal lineages of *D. obtusa* from each of two GPI genotypes (SF and SS), randomly selected from post-hatching adult females occupying the same temporary pond. These large, healthy females with large, early season broods were collected immediately after the pond refilled in autumn, and while the population was in Hardy-Weinberg equilibrium and beginning to reproduce parthenogenetically under the renewed favourable conditions of the cooler season. We therefore expect high genetic variation within the population. The experimental outcomes suggested some phenotypic expression of genetic variation in relation to the GPI genotype that was factored into the analysis. Most variation between genetically uniform clones was apparent at weeks 6 and 9 of the experiment (Figs. 3, 4, variation between grey bars), and at these times the SS genotype was generally outperforming SF. Previous work has shown associations between enzyme genotypes and fecundity in *D. magna* (Young 1979), suggesting that the enzyme GPI could be linked to genes controlling

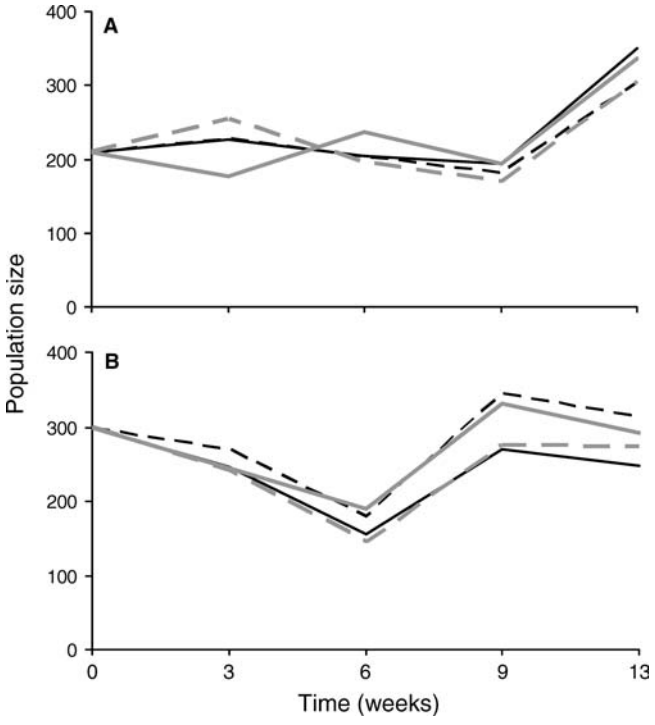

Fig. 6 Estimated number of *Daphnia* per population at 3, 6, 9 and 13 weeks, depending on initial size of invading group (10 = top graph, 100 = bottom), Mode of invader (genetically diverse *black lines*, uniform *grey lines*), and Genotype of invader (SF *broken line*, SS *solid line*). Each line is the back-transformed mean of four replicates

life history traits relevant to competition. Nevertheless, the outcome of competition consistently showed that the genetically diverse population could successfully invade and successfully resist invasion by genetically uniform populations regardless of the GPI genotype.

The invasion events manipulated in this experiment may reflect those that occur in nature. Obligately and cyclically parthenogenetic forms of *D. pulex* exist sympatrically across pond systems in North America (Hebert et al. 1988; Lynch et al. 1989). An event involving large numbers of one reproductive form invading into an established population of the other may occur when neighbouring ponds overflow and mix in the rainy season. Invasion events involving a smaller number of individuals may occur in nature as diapausing eggs are naturally dispersed by mammals or migratory waterfowl (Crease et al. 1997). The present experiment realistically reflects these natural occurrences, since the genetically diverse and genetically uniform individuals were not physically separated and the limited supply of impure food mix allowed the competitors to forage on their own resource base. No treatments underwent dramatic booms or crashes, reflecting the long-term stability often achieved by natural populations (Gurney and Nisbet 1998; Murdoch et al. 1998).

Obligately parthenogenetic clones of *D. pulex* show much genetic diversity for protein-coding loci (Weider et al. 1987; Hebert et al. 1988), despite the previous belief that clonal species had little or no genetic diversity (Williams 1975). Such variation is likely to be the result of a combination of accumulated mutations and of obligately parthenogenetic clones arising on independent occasions from the cyclically sexual populations (Lynch 1984b; Crease et al. 1989; Lynch et al. 1989). Despite the high genotypic diversity observed over a wide geographic area, most individual ponds containing obligate parthenogens consist of only a few clones (Hebert et al. 1988). Therefore, low genetic variation for the asexual population would result in increased intraspecific competition relative to the more genetically diverse sexual population as was modelled in the present experiments. Furthermore, Lynch (1984a) and Lynch et al. (1989) have demonstrated genetic variation for life history characters in *D. pulex*, providing the potential for genetic variation in resource use that could determine the outcome of competitive interactions as outlined in the present study.

Explanations for the short-term advantage of sex have been classified into environmental and mutational models (West et al. 1999). The Red Queen is an environmental-based model where sex is thought to increase adaptation to a changing biotic and/or abiotic environment. The advantage of sex for evolving resistance to parasites, which themselves can evolve to overcome this resistance, is currently the most popular version of the Red Queen model because of the coevolutionary dynamics of this biotic interaction (West et al. 1999). However, as pointed out by Butlin et al. (1999) a similar advantage for sex could be equally applied to other biotic interactions such as predator–prey and competition since these interactions also imply coevolutionary dynamics. Our results have focused on the advantage to sexual reproduction of genetic variation yielding competitive release in the presence of a genetically uniform asexual population. As pointed out by Stearns (1990), it is likely that the adaptive significance of sex involves interactions between the genotype and its environment, where biotic interactions may form the most important aspect of any heterogeneous environment. Whether the biotic interactions induced by crowding are sufficient alone to explain the adaptive significance of sex will require further investigation.

Acknowledgements The authors thank S.J. Hawkins, L.W. Beukeboom and C.K. Kelly for comments on earlier drafts. NT and CPD acknowledge support from the UK Natural Environment Research Council, and DJI from the Natural Sciences and Engineering Research Council (Canada). The experiments described in this study comply with the current laws of the UK.

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