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Comparisons of life-history traits between clonal and sexual fish (*Poeciliopsis*: *Poeciliidae*) raised in monoculture and mixed treatments

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Summary

Clonal and sexual co-existence is common in a number of vertebrate taxa, even though the 'cost of sex' makes such co-existence theoretically unlikely. The frozen niche-variation (FNV) model explains this co-existence on the basis of differences in overall niche breadth and competition between clones and sexuals. In the present study I examined two predictions of the FNV model. First, I examined the prediction that genetically variable populations have higher relative fitness when compared with monoclonal populations by comparing the performances of clonal and outcrossed sexual strains of *Poeciliopsis* in monocultures at two densities. The prediction of increased overall productivity for the sexuals was verified, with net reproductive rates for the sexuals being between two and four times as high as the clones. Second, I tested the prediction that derived clones will successfully compete with their sexual progenitor(s) in the narrow range to which the clones are adapted, while the sexuals should co-exist because of their ability to use a wider range of resources than any single clone. I examined this prediction by comparing performance variables (e.g. growth, fecundity and survival) of each strain in pure culture with their partitioned performance from the mixed treatments. Clonal performance increased in mixtures compared to monocultures, as expected. However, the expectation that the sexual's performance would be less affected by mixtures than the clones' performance, was not met. The sexuals had reduced growth and fecundity on a par with the increase in both variables in the clones. Therefore, support for the FNV model was mixed. Although the performance in monocultures suggests that the sexuals have a wider niche breadth than the clones, performances in mixtures do not indicate such a relationship. Switching of behaviours or resource-use patterns between mixed and pure cultures may have caused the equivocal results.

Keywords: maintenance of sex, unisexual reproduction; frozen niche-variation model; growth, survival and reproduction

Introduction

Sexual reproduction is the primary mode of genetic inheritance in vertebrates. In fact, sexuality is so entrenched in vertebrates that the only known cases of clonal reproduction occur in interspecific hybrids, in which the normal meiotic process has been disrupted. There are 74 known clonal hybrid vertebrates, which include clonal fish, amphibians and reptiles (for a complete list, see Vrijenhoek *et al.* (1989)). Extensive genetic analyses of many of these clonal–sexual complexes have usually revealed multiple clonal genotypes within these complexes and it appears that most of this variation is the result of multiple hybridization events (Parker and Selander, 1976; Vrijenhoek *et al.*, 1978; Dessauer and Cole, 1989; Goddard *et al.*, 1989). In the most well studied of these complexes, the clonal forms have been shown to co-exist with one or both of their sexual progenitors for dozens of generations (Vrijenhoek, 1984). In fact, some

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clones of the unisexual fish, *Poeciliopsis* may have persisted for as many as 100 000 generations (Quattro *et al.*, 1992). Co-existence between clones and their sexual progenitors is of interest because a number of theoretical models assume that clonal reproduction has a 2-fold advantage relative to sexual reproduction (Williams, 1975; Maynard Smith, 1978) and, thus, predict that co-existence is unlikely. The obvious question is: what factors combine to allow the co-existence of clones and sexuals in these complexes?

Three major hypotheses have been forwarded to explain co-existence in clonal–sexual vertebrate complexes. The first assumes that hybrid clones express phenotypes intermediate to both sexual progenitors and, thus, are well suited for intermediate niches (Schultz, 1977). In this scenario, niche partitioning would be facilitated by the intermediate phenotypes produced through hybridization between two sexual species. There are three major problems with this hypothesis. First, this model ignores the 2-fold 'cost of sex' mentioned above and therefore assumes that sexuals are superior competitors in their 'niche space.' Clones must occupy the niche space that is under exploited by the sexuals to co-exist. The question is therefore reversed. We should not ask, 'What allows clones to co-exist with sexuals?', but rather, 'What allows sexuals to overcome the 2-fold greater reproductive efficiency of the clones?'. The second problem concerns clonal diversity. If clones co-exist with sexuals because they occupy niche space intermediate to both sexuals, then the ubiquitous clonal diversity in clonal–sexual vertebrate systems is either neutral or clones must partition the possibly narrow niche space within the 'intermediate niches'. Ecological work in the *Poeciliopsis* system has shown that electrophoretic differences among clones is associated with ecological differences (Schenck and Vrijenhoek, 1986, 1989; Wetherington *et al.*, 1989; Weeks *et al.*, 1992) and, thus, that much of the electrophoretic diversity is not neutral. This ecological work has also revealed that ecological and behavioural differences among clones is extensive and, thus, underscores the third problem with the intermediate niche hypothesis: phenotypic differences among clones and between clones and their sexual progenitors clearly shows that clones are not constrained to express 'intermediate' phenotypes (Vrijenhoek, 1979, 1984; Schenck and Vrijenhoek, 1986; Wetherington *et al.*, 1989; Weeks *et al.*, 1992). Clonal phenotypes can be either more extreme than both sexuals, as in the case of predatory efficiency (Weeks *et al.*, 1992), or well within the range of a co-existing sexual, as in the case of growth, reproduction and offspring size at birth (Wetherington *et al.*, 1989; Weeks and Gaggiotti, 1993). Clearly, the notion that hybrid clones necessarily express intermediate phenotypes and therefore occupy 'intermediate niches' does not explain the complexity of the naturally occurring clonal–sexual complexes so far examined.

The second major hypothesis for clonal–sexual co-existence in vertebrate systems concerns the requirement of a sperm source for successful reproduction in many hybrid clones (Moore and McKay, 1971; Moore, 1984). Most clonal vertebrates require either the physical stimulation of eggs by sperm for initiation of development (as in 'gynogenetic' species) or actually require the genetic material from a sperm, only to discard this genetic material during egg production (as in 'hybridogenetic' species; see Schultz (1977) for a discussion of both modes of reproduction). Therefore, clones that drive both sexual progenitors extinct will also be doomed to extinction (Moore and McKay, 1971).

Frequency-dependent mating strategies in clonal–sexual complexes, whereby males fertilize clones only when clones are rare, can stabilize co-existence (Moore and McKay, 1971). The conditions for such frequency dependence have been described in one clonal–sexual complex in *Poeciliopsis* (McKay, 1971). However, there are two principal problems with the frequency-dependent hypothesis. First, the model does not account for the co-existence of clonal and sexual vertebrates in those complexes where sperm is not required for reproduction, as in the case of *Cnemidophorus* (Cuellar, 1971). Second, the model also does not adequately explain clonal

diversity. Clonal frequency is predicted to be approximately 80% or greater in clonal–sexual populations, regardless of the number of clones present (Moore and McKay, 1971). However, clonal frequency has been shown to be correlated with clonal diversity (Vrijenhoek, 1984). Clonal frequency ranges from below 10% in monoclonal pools (well below the predicted 80%) to 90% in multiclonal pools (Vrijenhoek, 1984). The frequency-dependent model, therefore, does not adequately explain either the low levels of clones in monoclonal pools, nor the correlation of clonal diversity with clonal abundance.

The third major hypothesis, the frozen niche-variation (FNV) model (Vrijenhoek, 1979, 1984), explains clonal and sexual co-existence by assuming that the phenotypic distribution of sexual populations is wider than the corresponding distribution of a monoclonal population because of genotypic variation among sexual individuals. Clones are expected to out-compete sexuals for the narrow range of resources to which they are best adapted, because of their 2-fold reproductive advantage. However, the sexual lineage is expected to persist because its wider niche breadth allows it to use resources unavailable to the monoclonal population. Sexual-clonal co-existence can be destabilized by increasing the number of distinct clones to the point where the clonal assembly eclipses the sexual niche (Vrijenhoek, 1979, 1984; Weeks, 1993) or, alternatively, by the evolution of ecologically 'generalist' clones (Baker, 1965; Maslin, 1968; Jaenike *et al.*, 1980; Lynch, 1984). The FNV model is attractive because it explains the association of multiple clones and higher clonal abundance: the greater number of ecologically distinct clones can more efficiently usurp portions of the sexuals' niche space and, therefore, should increase relative clonal abundance (Vrijenhoek, 1979, 1984; Weeks, 1993). Therefore, since the FNV model can explain both clonal diversity and the correlation of clonal diversity and abundance, it is the best candidate of the three to explain clonal-sexual co-existence in vertebrates.

The FNV model has two critical assumptions. First, it is assumed that disruption of meiosis in a progenitor sexual species, either by interspecific hybridization or by mutations, 'freezes' a single genotype and its associated range of phenotypic expression. The recurrent production of clones, either through hybridization or mutation, has abundant empirical support in natural clonal populations (Uzzell, 1964; Lowe and Wright, 1966; Hewitt, 1975; Uzzell and Darevsky, 1975; Parker and Selander, 1976; Schultz, 1977; Vrijenhoek, 1979; Jaenike *et al.*, 1980, 1982; Turner, 1982; Harshman and Futuyma, 1985; Lowcock *et al.*, 1987; Innes and Hebert, 1988; Dessauer and Cole, 1989; Goddard *et al.*, 1989). The second assumption is that the sexual ancestor(s) have considerable genetically-determined phenotypic variability for ecologically relevant traits, thereby allowing the freezing of ecologically distinct clones. This assumption has been thoroughly addressed using clonal and sexual populations of *Poeciliopsis*. Wetherington *et al.* (1989) found considerable between-individual genetic variation for size at birth, juvenile growth and early reproduction among 'hemiclones' (i.e. hybridogens) that were artificially produced from two *Poeciliopsis monacha* populations. Schenck and Vrijenhoek (1986, 1989) found both spatial and dietary differences between two clonal biotypes of *Poeciliopsis* in their native streams. Weeks *et al.* (1992) described differences in feeding behaviour between two naturally co-occurring clones, which corresponded with natural differences in diets in field-caught fish. These studies provide strong evidence for genetically determined, ecologically relevant phenotypic differences both within sexual progenitor species as well as among clonal lineages, and have verified the assumptions of the FNV model.

The next logical step in the progression of testing this model is to examine the prediction that derived clones will successfully compete with their sexual progenitor(s) in the narrow range to which the clones are adapted, but that sexuals will co-exist because of their wider niche breadth. Vrijenhoek (1979) has addressed this prediction by showing that sexuals comprise a smaller proportion of *Poeciliopsis* populations in streams where clonal diversity is high relative to

monoclonal streams. These correlations are suggestive of the predicted competitive interactions, but a manipulation experiment is necessary to thoroughly test the FNV model. An appropriate experiment requires comparisons of clones and their sexual progenitor(s) that have not had a long history of co-existence, thereby avoiding biasing clonal–sexual comparisons by using lines that are known to co-exist (Moore, 1975; Lynch, 1984). This consideration is especially important in the *Poeciliopsis* system, in which clones rely on sexual males for successful offspring production, which may artificially select for clones that successfully co-exist with sexual individuals (Lynch, 1984). The ultimate test would be to create a number of viable artificial clones from a *Poeciliopsis* population and then compare competitive interactions among clones and between clones and sexuals. Though a number of laboratory-synthesized clones of *Poeciliopsis* have been produced (see Wetherington *et al.*, 1987, 1989), there is no convenient way to distinguish among these clones morphologically or genetically, which eliminates the possibility of separating clones after mixture. Furthermore, many of these synthetic clones have reduced viability (see Wetherington *et al.*, 1987), which would tend to give an inflated advantage to the sexuals. The next best alternative is to identify natural, electrophoretically distinguishable clones and a sexual progenitor that is not relied upon as a sperm source for these clones to eliminate biasing in favour of co-existence.

I have used this second approach in the current experiment. I used two clonal strains of *P. monacha-lucida* and an outcrossed strain of one of their sexual progenitors, *P. monacha*, which is not the sperm source for either clone. These strains have peripatric natural distributions and the sexual population is thought to be descended from the progenitor population of both clones (Schultz, 1969; Vrijenhoek *et al.*, 1978; Quattro *et al.*, 1991). These strains were compared in controlled mixtures and densities in an artificial stream that mimicked natural habitat conditions. I attempted to incorporate a number of habitat characteristics that previous studies have implicated as important sources for niche partitioning: fast and slow current areas (Schenck and Vrijenhoek, 1986, 1989), live food and 'bottom' food (Schenck and Vrijenhoek, 1989; Weeks *et al.*, 1992) and high and low temperatures (Bulger and Schultz, 1979; Schultz and Fielding, 1989; E. Fielding unpublished manuscript). My goal was to quantify the effects of competition from a clonal assemblage on an outcrossed sexual progenitor population by measuring the life-history responses of these three lines in pure culture (at high and low densities) and in mixtures (at high densities).

Methods

Hybridization between *P. monacha* females and *P. lucida* males has given rise to the hybridogenetic diploid biotype *P. monacha-lucida* (Schultz, 1969). Hybridogenetic reproduction is 'hemiclonal' because only the haploid *monacha* (M) genome is transmitted, without recombination, to the eggs (Vrijenhoek *et al.*, 1978). Although the paternal *lucida* (L) genome is expressed in hybrids, it is discarded during oogenesis and replaced through fertilization by males of *P. lucida* (Schultz, 1969; Cimino, 1972). Two hybridogenetic clones (ML/VII and ML/VIII (Vrijenhoek *et al.*, 1978), hereafter referred to as C7 and C8) and an outcrossed sexual strain (*P. monacha* hereafter referred to as Pm) were used in this study. Distributions of these three strains overlap in the Rio Fuerte in Sonora, Mexico (Moore and McKay, 1971; Schenck and Vrijenhoek, 1986). Since the two clones do not depend on *P. monacha* for sperm, there is no constraint on these clones to co-exist with the sexuals in this system.

The experiment was designed to examine the relative performance of clones and sexuals in both monocultures and in mixtures. All treatments were started with juvenile fish between 3 and 21 days of age. To homogenize the developmental environment, juveniles were collected from 20

adult females that were kept in a flow-through aquatic incubator (25°C, 12l : 12d photoperiod) throughout their gestational period (see Wetherington *et al.*, 1989). Clonal parents were artificially inseminated with sperm from isogenic *P. lucida* males (strain S68–4 PC; Angus and Schultz, 1983), thereby standardizing the substitutable paternal genome and removing all between-individual genetically determined variation within a clonal strain (Wetherington *et al.*, 1989). Sexual females were naturally inseminated by *P. monacha* males in large rearing tanks. Juveniles were temporarily placed in three 356 l tanks until all 204 juveniles had been collected from each strain. Fish were weighed immediately before being randomly assigned to treatments. The experiment consisted of ten treatments in a randomized block design. The three 'pure-strain' treatments were reared at two densities (high = 24 and low = 12 fish per tank), and all combinations of strains were reared at the high density (Table 1). The entire design was conducted in two blocks of three replicates each.

The fish in all experimental treatments were fed brine shrimp nauplii, ground Purina trout chow, live chironomid larvae, live tubifex worms and live brine shrimp adults added once daily (see Weeks and Quattro, 1991). When live chironomid larvae were unavailable, frozen chironomid larvae were substituted. The same quantity of food was added to all treatments. Food was added through a series of half inch diameter, vinyl feeding tubes from a remote location so the fish would not be disturbed by the presence of a feeder.

Aquaria were housed in a greenhouse in a recirculating freshwater system that simulated small-scale heterogeneous conditions in natural Sonoran desert streams (Moore and McKay, 1971; Moore, 1975; Schenck and Vrijenhoek, 1986). Each 75 l glass aquarium had a standpipe allowing approximately 37 l of standing water. All aquaria had a single water input dropping onto an angled Plexiglass divider. This provided areas of fast current near the inlet and slow current near the outlet. The bottom of each aquarium was covered with 2 cm of sand. Three substrates were provided in different regions of each tank: (1) water-soaked straw, (2) pebbles (1–2 cm diameter) and (3) an aquatic moss. Recirculated water from all 30 experimental aquaria was collected in a 1125 l sump. Treatments ran for 70 days. The photoperiod was set at 15l : 9d by supplementing natural sunlight with broad-spectrum fluorescent light. Temperature was systematically varied throughout the experiment on a 3 week cycle using refrigeration and heating elements in the sump. To maintain a relatively high growth rate, temperature was left at the higher level (30°C) for 2 weeks followed by 1 week at the low level (20°C). Temperature differences among replicate tanks within a block were negligible (< 1°C) throughout the experiment.

On the seventieth day of all trials, survivors were sacrificed by placing them in icewater 45 min

Table 1. Experimental design

Treatment number	Designation	Treatment components
1	C7L	12 ML/VII
2	C8L	12 ML/VIII
3	PmL	12 *P. monacha*
4	C7H	24 ML/VII
5	C8H	24 ML/VIII
6	PmH	24 *P. monacha*
7	C7+C8	12 ML/VII + 12 ML/VIII
8	C7+Pm	12 ML/VII + 12 *P. monacha*
9	C8+Pm	12 ML/VIII + 12 *P. monacha*
10	C7+C8+Pm	8 ML/VII + 8 ML/VIII + 8 *P. monacha*

after feeding. The fish were counted, weighed and dissected. Eggs were categorized as either atretic (post-mature), mature or immature (see Wetherington *et al.*, 1989).

Statistical methods

Data were analysed using the SAS statistical package (SAS Institute, Inc., 1985). Male *P. monacha* exhibited determinate growth, whereas females were indeterminate. Therefore, to simplify the comparisons of sexual to clonal performance, between-line comparisons of growth were reported for females only.

In all analyses, the data were divided into two descriptive categories. First, the data were analysed by considering the overall performance of each treatment, with special emphasis on comparing the overall performance of clones to sexuals and comparisons of performances at high and low densities. The former comparison tests for increased sexual performance relative to monoclonal treatments. The latter comparison is important to show that the high-density treatments are food limited, which is useful for assessing the importance of resource partitioning in the mixed and monoculture treatments at the high density. In the second set of analyses, overall performance in the mixed treatments was decomposed into the performance of each component line. The decomposed performances of each of the three lines in mixed culture was then compared to that in pure treatments to examine which, if any, of the component lines benefited from being in mixtures.

Since treatments were applied to whole replicate tanks, all analyses consider only mean performances per tank. Growth per female was estimated by calculating mean increase in biomass per tank. The mean wet weight of all juveniles added to each tank was subtracted from the wet weight at the completion of the experiment for each fish. Mean growth per replicate tank was then computed by averaging this increase in biomass per female across all surviving females. Similarly, mean fecundity per replicate tank was calculated by counting the number of mature and atretic eggs per female and then averaging across all surviving females.

Univariate and multivariate analyses of variance were used to test for significant treatment effects on growth, fecundity and survival. Planned comparisons for differences in performance between both clones and the sexual strain were computed using orthogonal contrasts (SAS Institute, Inc., 1985). Statistical analyses of growth and egg production required log transformation of data and analyses of survival percentages required arcsin, square-root transformations to normalize distributions (Sokal and Rohlf, 1981). Density and strain treatments were considered fixed effects, whereas blocks were considered random. No block by treatment interactions were found for any of the fitness measures analysed. Therefore, block and treatment main effects were analysed using the overall mean square error in all analyses.

Results

Both growth and fecundity were heterogeneous among treatments (growth, $F_{9,49} = 24.81$, $p < 0.0001$; fecundity, $F_{9,49} = 15.44$, $p < 0.0001$). In the growth data, this heterogeneity was primarily due to the differences in size between the high- and low-density treatments (Fig. 1). Differences between treatments in egg production were more complex. Both *P. monacha* and C8 produced more eggs at low than at high density (Fig. 1). There was no significant difference in egg production between low and high density for C7. At the high density, the only significant difference in fecundity among treatments was between *P. monacha* and C7. There was significant heterogeneity among treatments for survival ($F_{9,49} = 2.27$, $p < 0.05$), due to the poor survival of C8 at low density (Fig. 1). The multivariate analysis of variance showed an overall difference

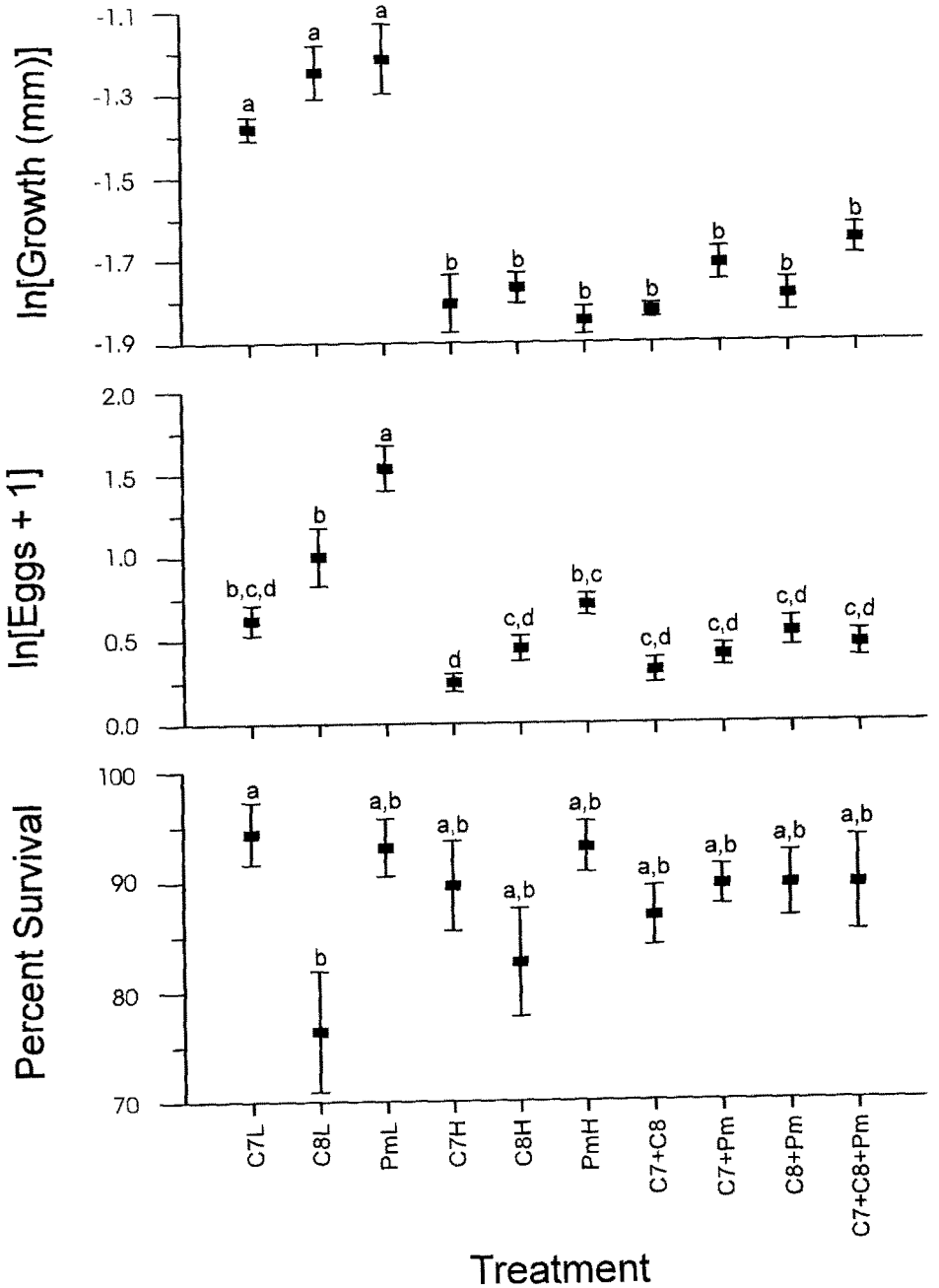

Figure 1. Growth, fecundity and survival for all ten treatments. Error bars portray one standard error of the mean. Means with the same letter designations are not significantly different at the $p = 0.05$ level (Ryan–Einot–Gabriel–Welsch multiple range test; SAS Institute, Inc., 1985).

Table 2. Pre-planned contrasts of performance variables measured in monocultures at high and low densities

Variable	df	Sum of Squares	*F* ratio	*p*-value
PmL versus C7L and C8L				
Growth	1	0.040	2.71	0.1060
Fecundity	1	2.103	36.46	0.0001
Survival	1	0.051	2.06	0.1572
MANOVA[a]	3	0.531	13.83	0.0001
PmH versus C7H and C8H				
Growth	1	0.014	0.98	0.3267
Fecundity	1	0.527	9.14	0.0040
Survival	1	0.068	2.72	0.1052
MANOVA[a]	3	0.700	6.72	0.0007
Error				
Growth	49	0.719		
Fecundity	49	2.826		
Survival	49	1.216		

[a] MANOVA values were calculated using Wilk's Lambda statistics.
Treatment designations are those outlined in Table 1.

between treatments when all three variables were considered simultaneously (Wilks' $\lambda = 0.050$, $p < 0.0001$).

Planned comparisons were used to determine if sexuals performed better than clones at the same density. *Poeciliopsis monacha* had higher fecundity than the mean of both clones in both high- and low-density treatments (Table 2). Though *P. monacha* had the highest growth at low density (Fig. 1), this difference was not significant. Also, although the survival of *P. monacha* was higher than C8 at both densities (Fig. 1), *P. monacha*'s survival was not greater than the mean of the two clones in these treatments. Consideration of all three variables simultaneously revealed a significantly greater mean performance for *P. monacha* relative to the clones at both low and high densities (Table 2).

To better understand the effects of mixtures on each strain, the overall performances were partitioned into the performances of each of the three strains. These partitioned performances were then compared to pure-strain performances at both high and low density. C7 showed significant heterogeneity in growth ($F_{4,24} = 25.55$, $p < 0.0001$) and fecundity ($F_{4,24} = 4.24$, $p < 0.01$) but not in survival ($F_{4,24} = 1.16$, $p > 0.35$). C7 grew significantly larger when in mixtures with *P. monacha* than in pure treatments at the same density (Fig. 2). C7's performance in mixtures was still significantly lower than when reared at the low density. A similar, but non-significant, increase in egg production was observed for C7 when in mixtures with *P. monacha* (Fig. 2). No differences in survival were observed among treatments at high density (Fig. 2).

Significant heterogeneity in both growth ($F_{4,24} = 21.68$, $p < 0.0001$) and fecundity ($F_{4,24} = 3.98$, $p < 0.05$) was found in the partitioned performance of C8. The major difference among treatments was between high versus low density (Fig. 3). However, C8 did reflect a similar pattern to that of C7: a tendency to grow larger and produce more eggs in mixtures with *P. monacha* than in pure treatments at the same density (Fig. 3). C8 did not survive better in mixed treatments ($F_{4,24} = 0.76$, $p > 0.50$; Fig. 3).

Similar to C8, the significant heterogeneity in both growth ($F_{4,24} = 10.43$, $p < 0.0001$) and fecundity ($F_{4,24} = 11.76$, $p < 0.0001$) found in the partitioned performance of Pm was mainly due

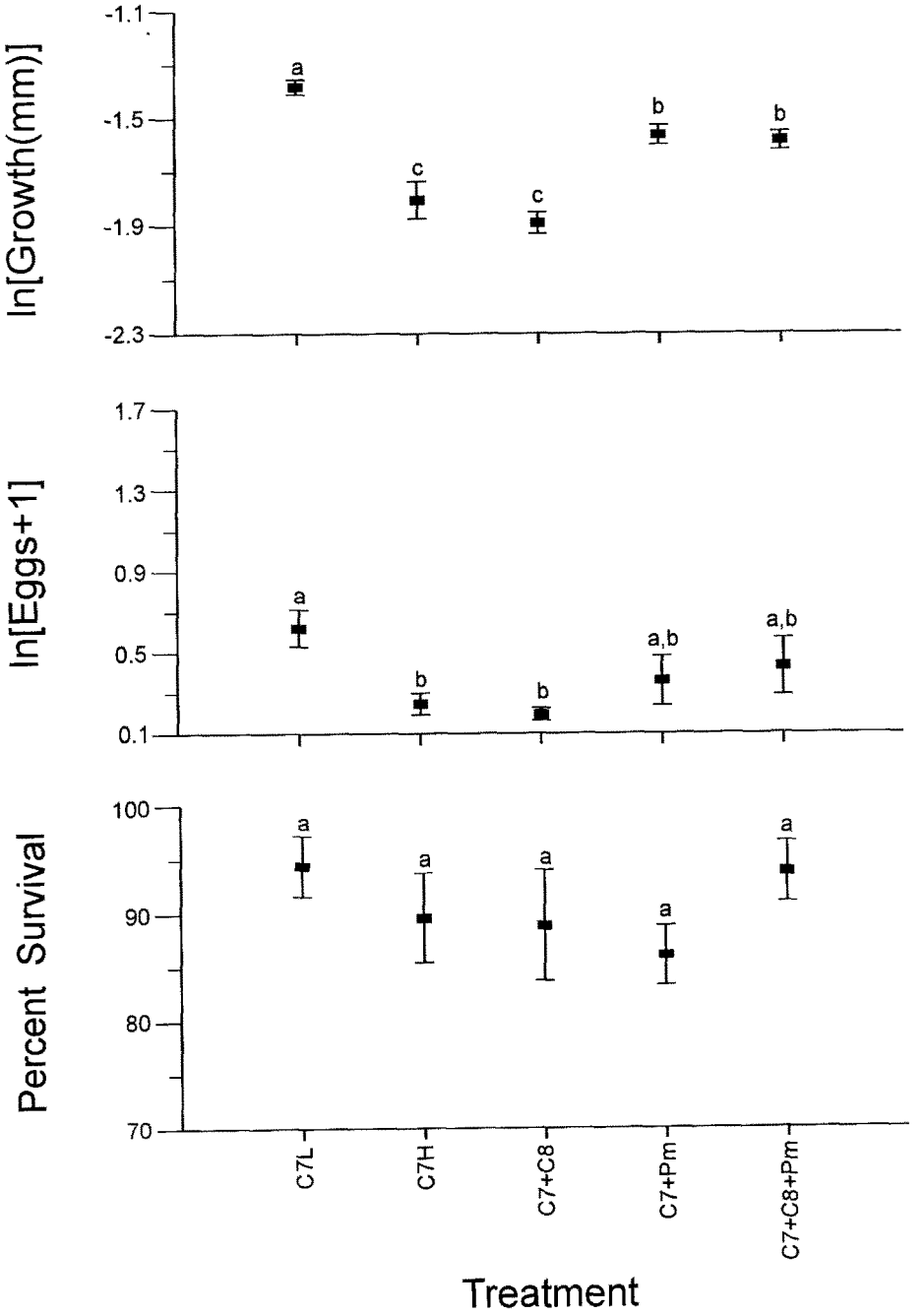

Figure 2. Partitioned growth, fecundity and survival for C7 in pure and mixed treatments. Error bars portray one standard error of the mean. Means with the same letter designations are not significantly different at the $p = 0.05$ level (Ryan–Einot–Gabriel–Welsch multiple range test; SAS Institute, Inc., 1985).

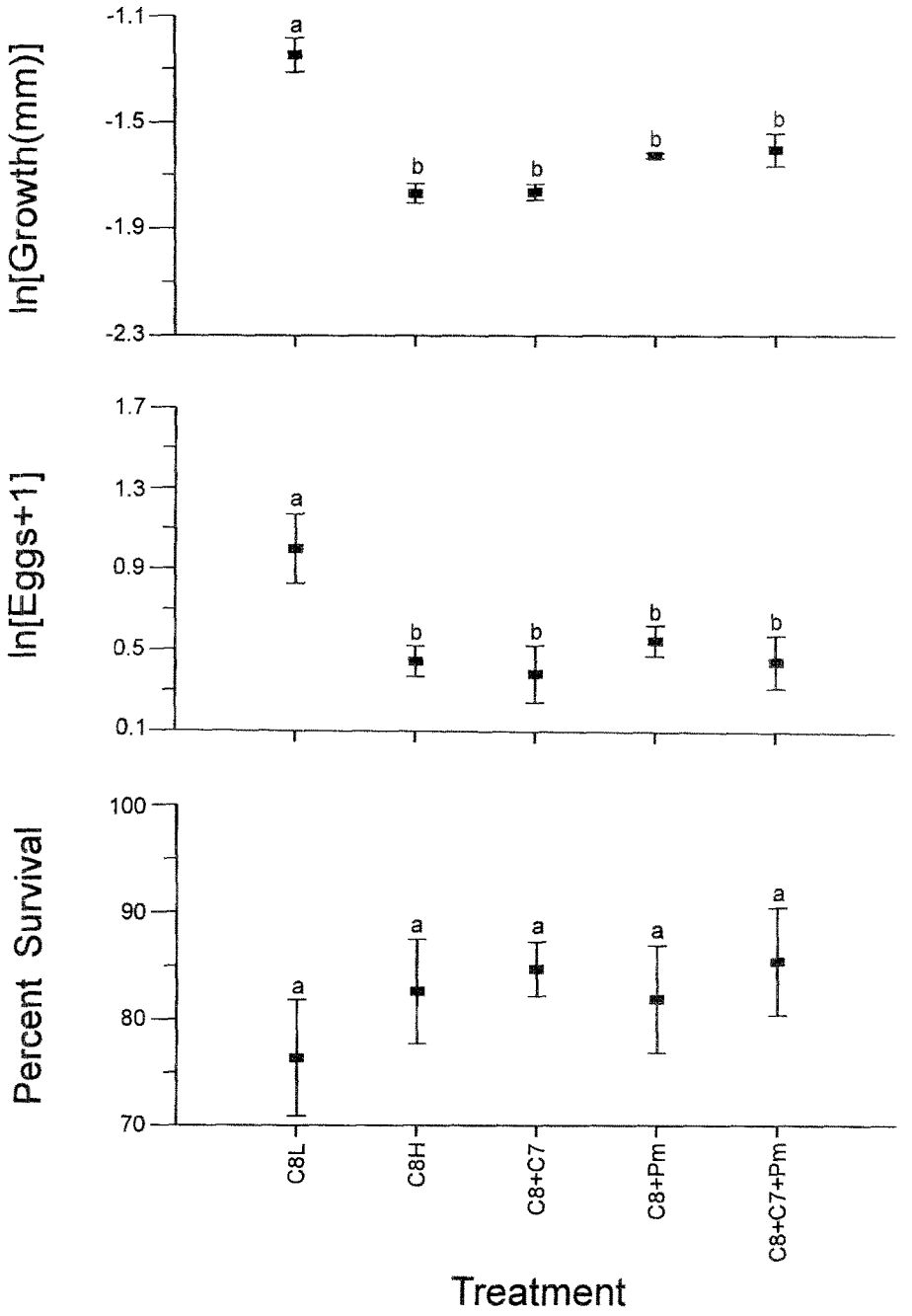

Figure 3. Partitioned growth, fecundity and survival for C8 in pure and mixed treatments. Error bars portray one standard error of the mean. Means with the same letter designations are not significantly different at the $p = 0.05$ level (Ryan–Einot–Gabriel–Welsch multiple range test; SAS Institute, Inc., 1985).

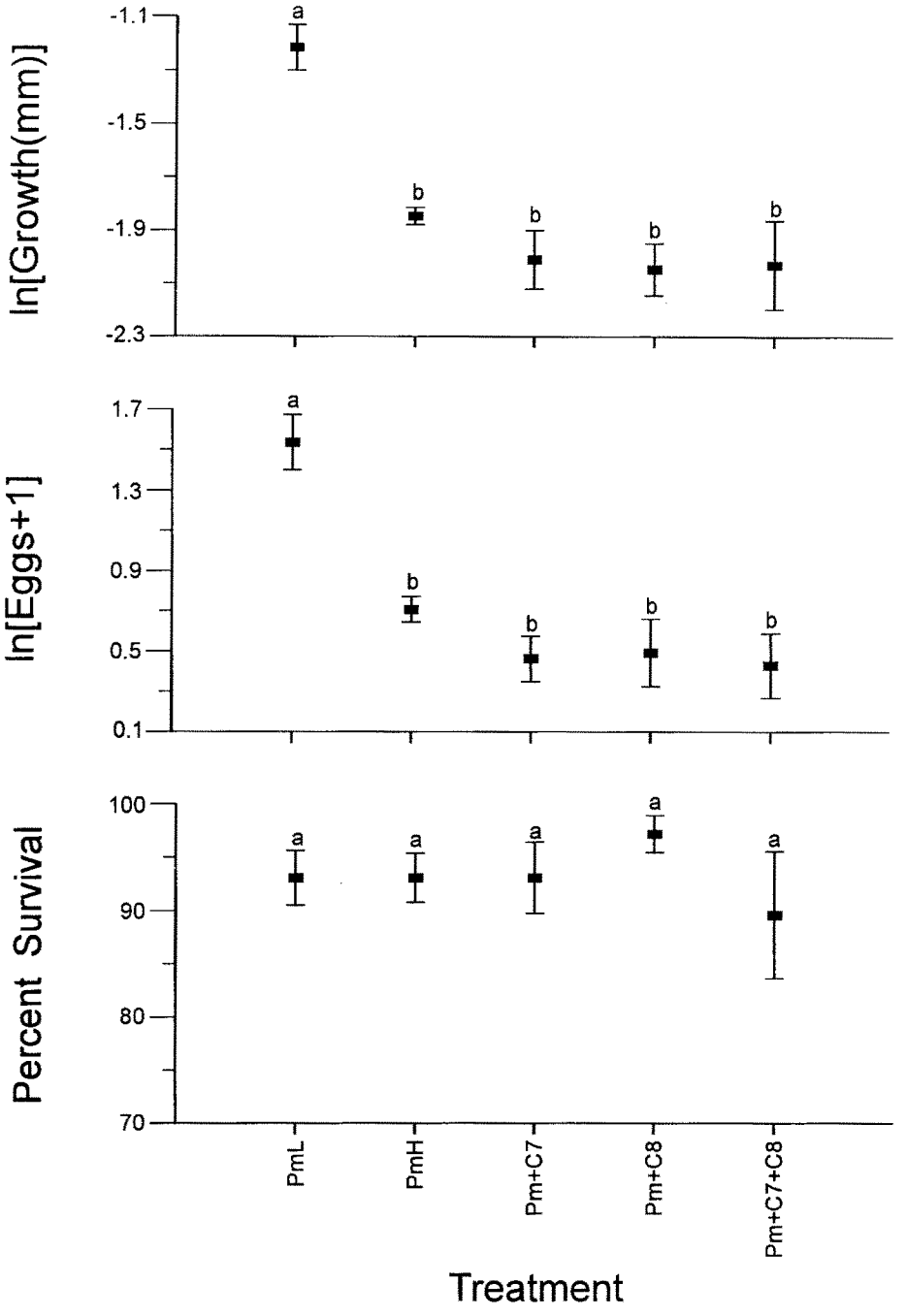

Figure 4. Partitioned growth, fecundity and survival for Pm in pure and mixed treatments. Error bars portray one standard error of the mean. Means with the same letter designations are not significantly different at the $p = 0.05$ level (Ryan–Einot–Gabriel–Welsch multiple range test; SAS Institute, Inc., 1985).

to performance differences among density treatments (Fig. 4). There were no significant differences in any fitness correlate for *P. monacha* in mixture versus pure-strain treatment at the high density (Fig. 4). However, there was a consistent trend of reduced growth and egg production for individuals in all three mixed treatments relative to pure-strain treatments (Fig. 4). As in the clonal strains, there were no significant differences among treatments for survival ($F_{4,24} = 0.43$, $p > 0.75$).

Discussion

Testing the predictions of the frozen niche-variation (FNV) model requires comparing the performance of outcrossed sexuals with one or more viable, derived clones in monocultures and in mixtures. Two general predictions of this model can be tested.

(1) An outcrossed sexual population should outperform a monoclonal population in a heterogeneous environment because the former can use more of the available niche space than the latter.

(2) Clones should successfully compete with the sexuals in the narrow range to which the clones are adapted, but the sexuals should persist because of their ability to use resources underutilized by the clones (Vrijenhoek, 1979, 1984).

To date, these predictions have been supported through laboratory and field research on sexual and clonal *Poeciliopsis* (Vrijenhoek, 1979, 1984; Schenck and Vrijenhoek, 1986, 1989; Wetherington *et al.*, 1987, 1989; Weeks *et al.*, 1992), though no previous study has successfully manipulated clonal and sexual populations in controlled mixtures (however, see Moore, 1975).

The goal of the present study was 2-fold. First, I examined the prediction of the FNV model that genetically variable populations have higher relative fitness when compared with monoclonal populations. Second, I quantified the effects of both clonal strains on the sexuals by comparing strain performance in mixed versus pure cultures. This second comparison addresses the FNV model's prediction that derived clones will successfully compete with their sexual progenitor(s) in the narrow range to which the clones are adapted, while the sexuals should co-exist because of their ability to use a wider range of resources than any single clone. I will examine each of these predictions separately below.

Performances in monocultures

Comparisons of overall performance of the sexual relative to the two clonal strains at high and low density verified the prediction that sexual strains outperform clones in a heterogeneous environment. *Poeciliopsis monacha* had higher mean fecundity than either clonal strain and had higher survival than one clonal strain (C8), without a corresponding trade-off in other performance variables. Using the product of mean survival with mean fecundity as a proxy for net reproductive rate (R_0), *P. monacha* had a mean R_0 2.2 times higher than C8 and 4.0 times higher than C7 in monocultures. These values are larger than the 2-fold greater performance necessary for sexuals to be resistant to clonal invasion. However, a number of factors make this simple comparison inconclusive.

First, the current experiment has taken only a snapshot of early productivity and therefore there is no basis upon which to compare total lifetime fitness. It is possible that *P. monacha* has higher investment in early reproduction and, thus, a comparison across the total lifespan may reduce the apparent advantage of the sexuals. Second, the difference in overall performance was negatively density dependent. The R_0 calculated above was, on average, 3.3 times higher for the sexuals than the clones at the low density, but only 2.9 times higher at the high density. Several

other studies have shown comparable density-dependent patterns of relative strain productivity. In experiments with sweet vernal grass, *Anthoxanthum odoratum*, sexually produced offspring survived better and had higher fecundity than clonally propagated tillers of a corresponding developmental stage under field conditions (Ellstrand and Antonovics, 1985; Kelley, 1989), though the relative advantage of sexuals was mitigated at higher densities. Differences in performance of mixed relative to pure-line treatments of inbred strains of shrimp (*Triops longicaudatus*) were greater at low and intermediate densities than at a high density (Weeks and Sassaman, 1990). Similarly, sexual *Artemia* out competed parthenogenetic strains in high-food treatments, but not in low-food treatments (Browne, 1980; Browne and Halanych, 1989). Studies on plants have also shown differences between strains to be more apparent at lower rather than higher densities (Heywood and Levin, 1984; Goldberg, 1988). This pattern of density dependence is opposite to that expected by ecological models of sex, which assume that the advantage of sex increases with increasing resource competition (Bell, 1982). It is possible that niche partitioning might be inefficient at reducing competition when overall population densities are extremely high. Therefore, the relative advantage of sexuals may depend on the levels of resource competition in their natural habitats. To test this possibility, one would need to set up mixed treatments similar to those used herein at a series of densities and note whether 'strain facilitation' (Bell, 1982) was more obvious at the lower densities.

Finally, the above comparison of relative productivity does not consider the interaction of clones with sexuals. Comparisons of mixed relative to pure treatments show that the performances in monocultures were very different from those in mixtures, as I shall examine below.

Performances in mixtures

To explore further the relative performances of clones and sexuals, I compared the partitioned productivities of the derived clonal lineages to that of the sexuals to note if clones can successfully compete with their sexual progenitor(s) in the narrow range to which the clones are adapted (Vrijenhoek, 1979, 1984). Multiple clones should co-exist if each can exploit a different subset of the environment. Clones and sexuals should co-exist if the multiclonal niche does not completely overlap that of the sexuals (Vrijenhoek, 1984; Weeks, 1993). Several studies on *Poeciliopsis* indicate these predictions are correct. A number of field collections revealed a negative correlation of the number of distinct electrophoretic clones and sexual abundance (Vrijenhoek, 1979, 1984). This relationship suggests that multiclonal assemblages do successfully compete for a portion of the sexuals' niche, thereby reducing the overall proportion of sexuals. Microhabitat sampling of clonal–sexual assemblages in Mexico has revealed further evidence of niche segregation among clones and sexuals (Schenck and Vrijenhoek, 1986, 1989). Gynogenetic triploid clones were more frequently collected drift feeding in currents, whereas sexual *P. monacha* were found more frequently feeding in pools (Schenck and Vrijenhoek, 1986). These differences in spatial use were also correlated with differences in diet (Schenck and Vrijenhoek, 1989). Niche segregation based on spatial heterogeneity also appears to be important in other clonal–sexual *Poeciliopsis* complexes (Vrijenhoek, 1984; Schenck and Vrijenhoek, 1986). Differences in predation efficiency, which also correlate with dietary preferences in the field, have been documented between two hybridogenetic clones and their sexual ancestors (Weeks *et al.*, 1992). These differences may facilitate co-existence among both clonal and sexual lines.

Though these studies are all consistent with the predictions of the FNV model, no study has specifically compared the fitness of clones and sexuals in monocultures and in mixtures. These comparisons are necessary to show unequivocally that clones can successfully sequester a portion of the sexuals' niche, but that sexuals can co-exist because of their wider niche breadth. Predicted fitness responses to mixtures differ between sexual and clonal lines. Competition among clonal

individuals in monocultures is predicted to be intense and, thus, clonal members should experience a release from competition in mixed treatments, resulting in increased productivity. Conversely, competition in sexual monocultures should be lower than in mixtures with clones which are usurping a portion of the sexual's niche. However, if the niche width of the clones is truly narrower than that of the sexuals, a 1 : 1 mixture of the clones with sexuals should reduce the between-individual competition to a greater degree for the clones than increase competition for the sexuals, resulting in an asymmetrical response to mixtures.

Clearly, the clones benefited from being in mixtures with sexuals relative to monocultures at the same density. Both clones had a trend of increased growth and fecundity in mixtures with *P. monacha*. Mean clonal growth increased by 21% and fecundity by 50% in 1 : 1 mixtures with sexuals and increased by 21 and 61%, respectively, in the three-way mixture. Conversely, the sexuals did poorly when mixed with clones, exhibiting decreases in both growth and fecundity, though these decreases were not significant. Mean growth of sexuals was reduced by 20% and fecundity by 40% in 1 : 1 mixtures and by 13 and 65%, respectively, in three-way mixtures with clones. Therefore, the predicted competitive response of sexuals and clones in mixtures and the successful usurping of niche space by the clones was verified in these treatments. However, the expected asymmetric response to 1 : 1 mixtures was not borne out in this experiment. These results suggest that the overall niche breadth of the sexuals in this environment was not large enough to produce the expected asymmetry in performance.

An obvious problem between the current results and the FNV model concerns the lack of evidence for reduced competition between C7 and C8 in mixtures. Differences in behaviour (Keegan-Rogers and Schultz, 1984; Weeks *et al.*, 1992), diet (Weeks *et al.*, 1992), physiology (Bulger and Schultz, 1979; Schultz and Fielding, 1989), and small-scale spatial heterogeneity (Schenck and Vrijenhoek, 1986), combined with the apparent long-term co-existence among these strains in Mexico (Vrijenhoek, 1984; Quattro *et al*, 1991) suggests that some form of niche partitioning facilitates co-existence. Yet, there was no evidence that mixtures of these lines in the artificial stream reduced competition for either strain. It is possible that the restricted nature of the artificial stream may have missed one or more important variables allowing niche separation. As mentioned above, larger-scale spatial segregation than was possible in the laboratory might be necessary for co-existence of these strains (Schenck and Vrijenhoek, 1986).

The current experiment provides limited support for the frozen niche-variation model for the co-existence of clonal and sexual vertebrates. Sexual monocultures outperformed clonal monocultures at both high and low densities, presumably due to reduced between-individual competition in the former. The FNV model's prediction that the clones should successfully compete with the sexuals in the range to which the clones are adapted was also verified: considering the clones have a 2-fold reproductive advantage (because they do not produce males; see Maynard Smith, 1978), their successful competition for resources and space in the current study should ensure successful sequestering of a portion of the sexual niche space, as predicted by the FNV model. It is unclear whether niche partitioning in natural environments would allow this combination of clones and sexuals to co-exist successfully, given a sperm source for the clones. Clearly, the notion that the negative correlation of the proportion of sexuals with the number of distinct clones in *Poeciliopsis* is caused by the clones successfully usurping the sexual's niche space (Vrijenhoek, 1979, 1984) is strengthened by the results of the current experiment. Therefore, these results reinforce the notion that stable sexual-clonal co-existence should be confined to populations comprised of a few specialized clones and a generalized sexual species (Vrijenhoek, 1979, 1984; Weeks, 1993).

However, there are a number of questions relevant to the FNV model that remain to be addressed. Foremost among these questions is whether *P. monacha* actually exhibits a wider

niche breadth than the clones. The results of the current experiment are ambiguous regarding niche breadth. The productivity measures taken from all three strains in monocultures suggest that the sexuals were better able to use the heterogeneous environment than the clones and were thus more productive. Yet, the results in mixtures did not show productivity results that were consistent with the assumption of increased niche breadth for the sexuals. An obvious question is whether the niche breadths of the sexuals and the clones were different in monocultures, but somehow converged in mixtures. Differences in behaviours or resource use of the clones, the sexuals or both could have caused such a convergence in niche breadths in the mixed treatments. I am currently investigating these possibilities by comparing behaviours and diets of these three strains in pure and mixed cultures. Hopefully, these additional data will facilitate the interpretation of the current results as well as provide further tests of the FNV model.

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