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No-choice mating experiments among six nominal taxa of the subgenus *Physella* (Basommatophora: Physidae).

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With 5 figures and 1 table.

Abstract.

Breeding studies have recently demonstrated no postzygotic reproductive isolation among several of the most common nominal species in the subgenus *Costatella* of the widespread freshwater pulmonate snail family Physidae. Here we extend these results to include six nominal taxa in the other major subgenus of North American physids, *Physella*. We established first generation lines from the type or near-type localities of *ancillaria* (A), *aurea* (V), *gyrina* (G), *microstriata* (M), *parkeri* (K), and *utahensis* (U). These were crossed in five no-choice experiments (AxV, GxV, GxK, GxM, and MxU) and reared along with corresponding incross controls, monitoring reproduction to the F_2 generation. Parental reproduction was not significantly delayed in any outcross experiment, and the production of hybrid F_1 progeny was confirmed in all outcrosses by allozyme markers. In no experiment was parental fecundity, F_1 viability, or F_1 fertility significantly depressed below both incross controls. However, control crosses displayed great variation in survivorship and reproductive schedule under our standard culture conditions. Both *P. parkeri* and *P. ancillaria* showed strikingly delayed maturation, the former with reduced survivorship and fecundity, the latter with good survivorship until a burst of semelparous reproduction. Although our experiments yielded no evidence of postzygotic reproductive isolation among any of the six nominal taxa we tested, a great deal of apparently heritable life history divergence was nevertheless in evidence.

Introduction.

The Physidae is a worldwide family of freshwater pulmonate snails characterized by high reproductive output, phenotypic plasticity, and adaptability to a broad range of environmental conditions. Their diversity of reproductive response, which includes self-fertilization, mixed mating, and outcrossing in either gender, together with their ease of laboratory culture, has made them model organisms for the study of sex allocation (WETHINGTON & DILLON 1991, 1993, 1996, 1997; DILLON, EARNHARDT & SMITH in press). Their center of taxonomic diversity is North America, where 36 species and 43 subspecies have been allocated into two genera of the subfamily Aplexinae and two genera (with four subgenera) of the subfamily Physinae (BURCH & TOTTENHAM 1980).

Recently it has become apparent that the number of physid species has been overestimated. DILLON & al. (2002) easily hybridized three of the most common nominal species in the Physine subgenus *Costatella*: European *Physa acuta* (DRAPARNAUD 1805), *P. heterostropha* (SAY 1817) from the American south and east, and *P. integra* (HALDEMAN 1841) from the American north and Midwest, failing to detect any delay in parental maturity or reduction in parental fecundity, hybrid viability, or hybrid fertility. When taken with previous observations demonstrating little prezygotic reproductive isolation within this group (WETHINGTON & al. 2000), and the absence of any consistent differences in allozyme frequency or mitochondrial sequence divergence (WETHINGTON & al., in review), a conclusion that *heterostropha* and *integra* are synonyms of *acuta* would seem inescapable. Our more recent experiments have uncovered no reproductive isolation between *P. acuta* and *P. virgata* (GOULD 1855), the most common physid in the American west and southwest (DILLON & WETHINGTON, in prep). Ongoing surveys of mitochondrial sequence divergence suggest that many of the 14 American species of physids in the

subgenus *Costatella* (characterized by a one part penial sheath) may ultimately prove synonymous (WETHINGTON & LYDEARD, in review.)

Reproductive isolation is complete, however, between *P. acuta* and *P. gyrina aurea* (LEA 1838). *Physa gyrina* is a member of the Physine subgenus *Physella*, characterized by a two-part penial sheath. Reared in pairs with *P. acuta*, *P. gyrina aurea* failed to reproduce entirely, while *P. acuta* reproduced only by self-fertilization (DILLON, EARNHARDT & SMITH in press). Thus although there may be little biological basis for distinctions among many nominal species within physid subgenera, the subgenera themselves seem unambiguously distinguishable.

The standard no-choice experiments we have used in our laboratory to test for postzygotic reproductive isolation in physids follow the reproduction of paired snails reared in small disposable drinking cups. Equal numbers of control A pairs, control B pairs, and AxB outcross experiments are monitored through the production of viable F_2 hatchlings, with allozyme electrophoresis employed to verify the hybrid status of F_1 progeny. Evidence for reproductive isolation is gathered using four criteria: any delay in the age at which the AxB outcross pairs reproduce behind the two controls, any reduction in the number of eggs laid by AxB outcross relative to the two controls, any reduction in the viability of AxB hybrid eggs relative to control eggs (as measured by percent hatched) and any reduction of the reproductive success of the F_1 hybrids.

The purpose of the present paper is to report tests for postzygotic reproductive isolation among a broad sample a populations representing the subgenus *Physella*. If most of the species currently recognized in the subgenus *Costatella* ultimately yield to synonymy, might a similar situation exist among the 16 species and 18 subspecies of the subgenus *Physella* recognized in North America today?

The first species in the subgenus *Physella* to reach formal description was *P. gyrina* (SAY 1821), collected from Council Bluffs, Iowa. It includes among its subspecies the taxon *aurea* (LEA 1838), described from Hot Springs, Virginia, and 13 other subspecies and "morphs" primarily inhabiting the northern and western United States and Canada. Another widespread member of the subgenus *Physella* is *P. ancillaria* (SAY 1825), described initially from the Delaware River at Easton, Pa., but common throughout the northeastern United States and Canada. *Physa parkeri* (CURRIER 1881) is a larger animal bearing a broader shell with more angular shoulders, initially described from Houghton Lake, Michigan and generally restricted to colder lakes in the American Midwest. *Physa utahensis* (CLENCH 1925) is a similarly broad-shelled *Physella* species first described from Utah Lake, Utah, and ranging through mountainous areas of the America west. *Physa microstriata* (CHAMBERLAIN & BERRY 1930), described from Fish Lake, Utah, is characterized by an unusually high spired shell with a narrow body whorl.

Here we report the results of a series of five outcross experiments which, together with eight corresponding control crosses, have returned no evidence of postzygotic reproductive isolation among this sample of six nominal taxa from the subgenus *Physella*.

Methods.

Our sample of *Physa gyrina* (population G) was collected from its type locality, the Boyer River at the Route 183 bridge north of Council Bluffs, Iowa (41.537° N, 95.885° W) in July, 2001. We collected *Physa gyrina aurea* from its type locality in the town of Hot Springs, Virginia, approximately 100 meters downstream from the origin of naturally heated waters inside "The Homestead" resort (38.000° N, 79.832° W) in June, 2001 (V1) and again in May, 2002 (V2). Topotypic *Physa ancillaria* (A) were collected at the Route 248 bridge over the Delaware River at Easton, Pennsylvania in May, 2002 (40.692° N, 75.204° W). Our sample of *Physa parkeri* (K) was collected in June, 2001 at the University of Michigan Biological Station, Douglas Lake (45.565° N, 84.680° W), approximately 150 km north of the type locality for the species. *Physa microstriata* (M1 and M2) were collected from Fish Lake by Route 25 (38.557° N, 111.713° W) and *P. utahensis* (U) from Utah Lake at the State Park boat harbor (40.239° N, 111.738° W), both in Utah, in August 2001.

Our standard culture vessel was a transparent polyethylene 10 ounce drinking cup, which we filled with approximately 210 ml of aerated, filtered pond water and covered with a 95 x 15 mm polystyrene

Petri dish lid. The food was O. S. I. *Spirulina* Aquarium Flake Food, sold in pet stores primarily as a diet for herbivorous aquarium fishes. All experiments took place at room temperature, approximately 23°C.

We isolated ten wild-collected snails from each study population in separate cups, collected egg masses, and reared the offspring to 3 mm shell length (approximately three weeks post-hatching), with weekly water change. From these unrelated sets of ten wild-conceived but laboratory-born sibships were selected the parental generation for each experiment and its corresponding controls.

Controls were comprised of ten pairs of unrelated parents from within a population, for example A1xA2, A2xA3, ..., A10xA1 and B1xB2, B2xB3, ..., B10xB1. Experimental crosses were sets of ten cups paired across populations, for example AxB1, AxB2, ..., AxB10. Each pair of parents received a water change and fresh food every seven days, at which time the sides of the cup were inspected for egg masses. If egg masses were present, we counted all embryos and transferred the adults to a fresh cup. Eggs were monitored until hatching (generally about two weeks) and all viable, crawling F_1 juveniles were counted. Observation was terminated upon the death of either parent in a pair.

Any difference in the central tendency of age at first reproduction between an experiment and its control populations was tested pairwise by calculating a combined median, then comparing counts above and below that median using a Fishers Exact test. For statistical analysis of fecundity and F_1 viability, week 1 was set separately within each experiment or control as the first week in which eggs were laid by three or more pairs of parents. Egg production and hatching success were subsequently recorded for ten weeks. We compared the fecundity of each experiment to its controls using a two-way analysis of variance, week and population being the independent variables and embryos the dependent variable (Statistica release 5.5, StatSoft, 1994). Overall (ten-week) F_1 viability was compared between experiments and corresponding controls using analysis of covariance, with treatment the independent variable, viable hatchlings the dependent variable, and embryos the covariate. Post hoc tests were performed using Tukey's "highly significant difference" (HSD) tests for unequal sample sizes (SPJOTVOLL & STOLINE 1973). Leading (pre-maturity) zeros were not included in any ANOVA or ANCOVA, nor were post-mortem zeros included, although internal zeros (i. e., reproductive failure by mature, apparently healthy snails) were analyzed.

To assess the fertility of putative hybrid offspring, F_1 hatchlings (both control and experimental) were reared from each of three separate unrelated pairs to size 3 mm. These were paired in time series: one early pair from eggs laid around week 1, one middle pair produced around week 5, and one late pair produced around week 10, to yield nine F_1 pairs. So if the putative hybrid progeny were reared from pairs AB1, AB2, and AB3, they were crossed as AB1xAB2 early, AB2xAB3 early, AB3xAB1 early, AB1xAB2 middle, AB2xAB3 middle, ..., AB3xAB1 late. Nine pairs would likewise be constituted for controls A and B, and the total of 3x9=27 pairs of F_1 snails reared to adulthood for each experiment, with weekly feeding and water change. We recorded the date at which embryos and viable F_2 hatchlings were produced by each pair.

A larger sample of F_1 progeny from each experiment was reared to 4-5 mm shell length, at which time they were frozen in 100:1 of tissue buffer for analysis by allozyme electrophoresis. We have identified 12 enzyme-encoding loci at which allozyme variation is interpretable as the product of codominant alleles segregating in MENDELian fashion (DILLON & WETHINGTON 1994). These are aconitase (Acon), esterases (three loci: Est1, Est3, Est6), glucose phosphate isomerase (Gpi), isocitrate dehydrogenase (two loci: Isdh1 and Isdh2), leucine aminopeptidase (Lap), mannose phosphate isomerase (Mpi), phosphoglucomutase (two loci: Pgm1 and Pgm2), and 6-phosphogluconate dehydrogenase (6pgd). We used horizontal starch gel electrophoresis in an aminopropylmorpholine pH 6 buffer system to resolve allozyme variation at the Gpi, Isdh, and 6pgd loci, a Tris-Citrate pH6 buffer system for Acon, Mpi, and Pgm, and a TEB8 system for 6pgd, Lap, and Est. Details regarding our electrophoretic methods, including a description of our equipment and recipes for stains and buffers, have been previously published (DILLON 1992, DILLON & WETHINGTON 1995).

Our five experiments, with corresponding controls, were performed in three trials. Trial 1 took place in Charleston 9/2001-7/2002 and crossed *P. gyrina* from Council Bluffs to three other populations: *aurea*, *parkeri*, and *microstriata*. Thus there were four incross controls (G, V1, K, and M1) and three outcross experiments (GV, GK, and GM) for a total of 70 pairs of parents in trial 1, G serving as a control for

three experiments simultaneously. Trial 2 took place in Tuscaloosa 11/2001-7/2002 and crossed *microstriata* to *utahensis*. Two incross controls (U and M2) were compared to the UM outcross experiment, totaling 30 pairs of parents. Note that this second M control ("M2") involved ten pairs of *microstriata* parents different from those used for the M1 control in Charleston. Trial 3 took place in Charleston 6/2002-3/2003 and crossed *aurea* to *ancillaria*, as well as to *Physa acuta* in several experiments published elsewhere. We report the results of incross control A and outcross experiment AV here, comparing these data to a second *aurea* control ("V2") reanalyzed from DILLON, EARNHARDT & SMITH (in press).

Results.

GV Experiment.

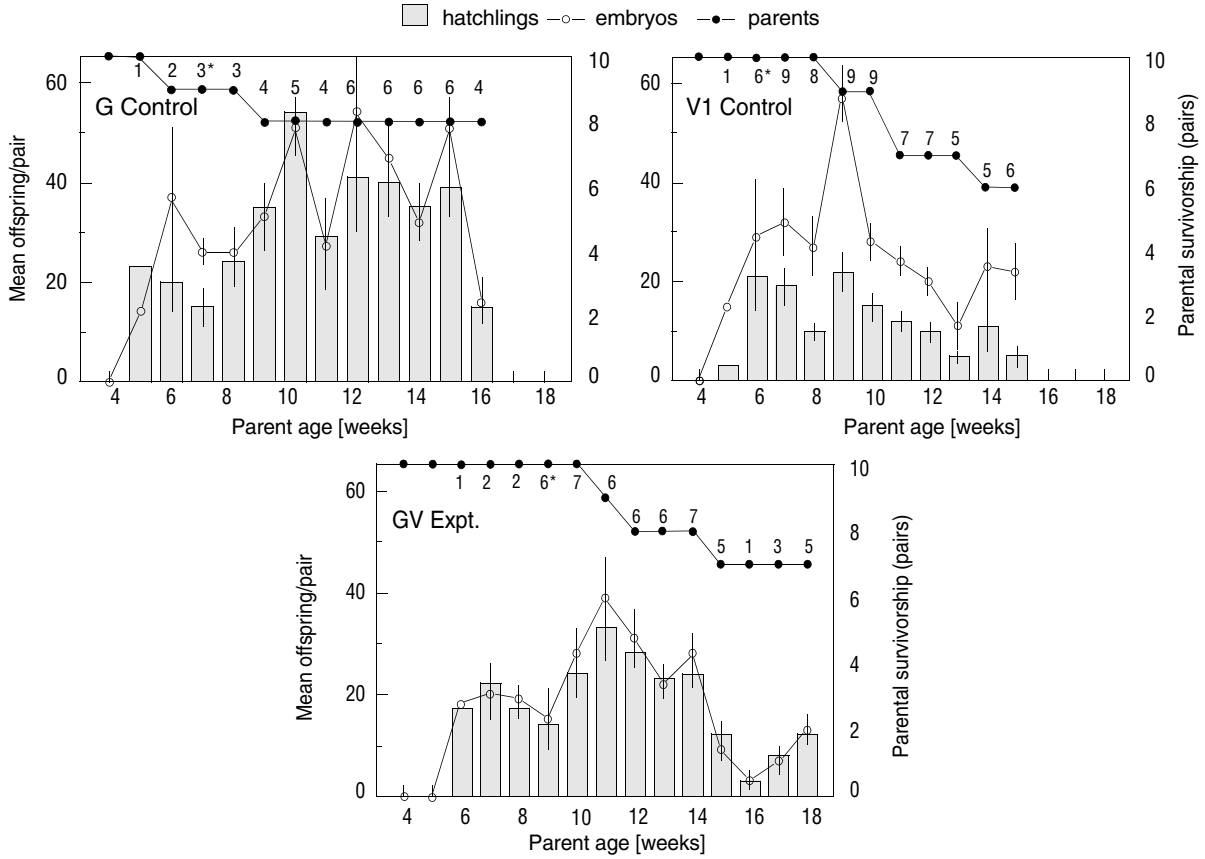

Fig. 1. Survivorship and reproduction as a function of parental age (weeks post hatching) for ten pairs of *Physa gyrina gyrina* (G control), ten pairs of *Physa gyrina aurea* (V1 control), and the GV outcross experiment. Bars are standard errors of the mean. The number of reproducing pairs is given with parental survivorship (right axis). Asterisks* denote week 1 for analysis of variance.

Parental survivorship, fecundity, and F_1 viability for the *gyrina* control, the first *aurea* control, and the *gyrina* x *aurea* experiment are compared in Figure 1. Three pairs of G control parents died without issue, two of which lived in excess of 18 weeks and seem to have been sterile. One GV pair died early in the experiment and one GV pair also seemed to have been sterile. Table 1 shows that the age at first reproduction of the 8 pairs of GV parents was 9 weeks, significantly delayed behind the V1 control (FISHER's exact $p=0.013$) but not different from the age at which the 8 pairs of G control parents reproduced. Two-way analysis of variance showed a highly significant difference in mean fecundity among these three treatments ($p=0.0006$). Post hoc tests showed that the fecundity of the GV control (20.4 embryos/pair/wk) was significantly below the 36.2 embryos/pair/week posted by the G control ($p=0.0005$) but not the V control ($p=0.069$).

Analysis of covariance returned a significant difference in the F_1 viability, the GV viability of 81.4% greater than the 45.0% posted by the V1 control ($p=0.035$) but not different from the G control. Protein electrophoresis revealed a fixed difference at the 6pgd locus between two populations. A sample of 23

putative hybrids from three different GV crosses proved to be entirely heterozygous, confirming the hybrid nature of the F_1 progeny. All nine F_1 pairs were fertile, producing viable F_2 progeny at a median age of 9 weeks – earlier than either pure control line (Table 1).

Table 1. Summary statistics on reproduction in 13 laboratory populations of *Physella*. Fecundity and yield of viable hatchlings are ten week totals per pair for all cases except the GK experiment and the K and A controls, which are totaled over 36 weeks.

	Pairs reproducing	**Week of first reproduction** (median, range)	**Total fecundity** (mean ± s.e.m.)	**Total hatchlings** (mean ± s.e.m.)	**F_1 viability**	**Week of F_2 hatch** (median, range)
V1 control	10	6, 5 - 9	215 ± 34.5	96.9 ± 17.3	45.0%	16, 7 - 21
GV experiment	8	9, 6 - 12	183 ± 33.6	149 ± 28.9	81.4%	9, 7 - 24
G control	7	8, 5 - 11	283 ± 36.4	231 ± 34.7	81.6%	11, 6 - 26
GM experiment	10	8.5, 5 - 10	222 ± 53.7	169 ± 45.0	75.3%	10, 3 - 13
M1 control	9	5.5, 4 - 10	188 ± 41.3	105 ± 28.7	55.6%	13, 10 - 23
GK experiment	6	8 - 32	83.5 ± 43.8	66.3 ± 43.6	77.7%	-
K control	5	5 - 32	104 ± 35.3	61.0 ± 27.2	58.7%	-
V2 control	9	11.5, 10 - 13	212 ± 31.3	71.8 ± 15.7	33.8%	-
AV experiment	7	12.5, 11 - 19	93.7 ± 18.1	45.1 ± 10.1	48.1%	-
A control	6	25 - 36	222 ± 85.8	62.5 ± 22.0	28.1%	-
M2 control	7	7.5, 7 - 8	184 ± 73.3	165 ± 64.5	89.7%	13, 7 - 16
UM experiment	10	7.5, 6 - 8	333 ± 58.9	236 ± 42.9	70.7%	12, 7 - 21
U control	10	7, 6 - 8	636 ± 114	518 ± 89.7	81.3%	7, 5 - 19

GM Experiment.

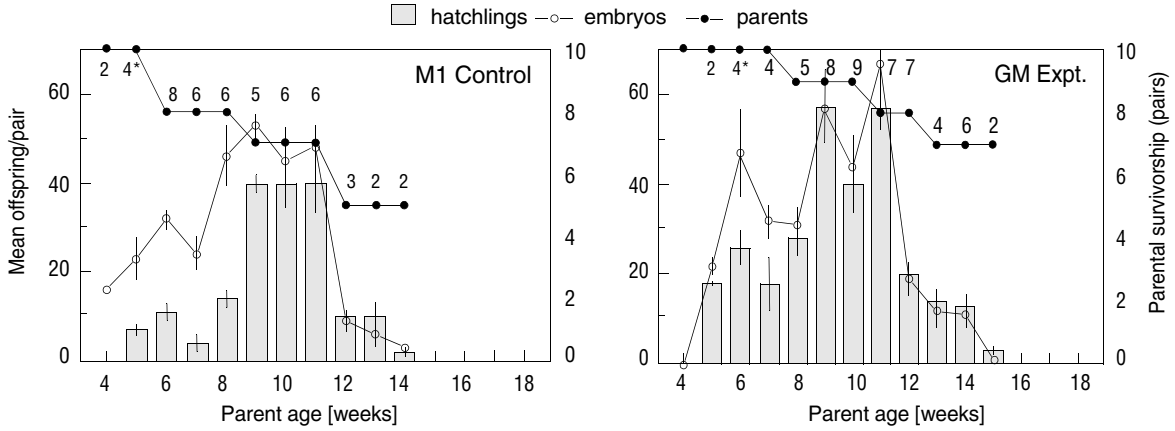

Fig. 2. Survivorship and reproduction as a function of parental age (weeks post hatching) for ten pairs of *Physa microstriata* (M1 control) and the GM outcross experiment. The corresponding *Physa gyrina* control (G) can be viewed in Figure 1. Bars are standard errors of the mean. The number of reproducing pairs is given with parental survivorship (right axis). Asterisks* denote week 1 for analysis of variance.

Figure 2 compares the parental survivorship, fecundity, and F_1 viability of the *gyrina* x *microstriata* experiment to its *microstriata* control, while the corresponding *gyrina* control may be viewed in Figure 1. All ten pairs of GM parents reproduced, while one pair of M control parents seems to have been sterile. The delay in reproduction posted by the GM parents (median=8.5 wks) behind that of the M1 control (median=5.5 wks) approached significance (FISHER's exact p=0.057), but was not significantly different from that posted by the G control. Two-way ANOVA returned no significant difference in ten week fecundity between the GM experiment and its corresponding controls (p=0.206).

Electrophoretic analysis revealed few differences in allozyme allele frequency between the pure control lines, although substantial polymorphism was present at several loci. We were able to confirm outcrossing in samples from three GM crosses – 22 GM5 offspring included 14 FF homozygotes and 8

FS heterozygotes at the 6pgd locus, 12 GM7 offspring included 8 FF homozygotes and 4 FS heterozygotes at the Gpi locus, and 14 GM9 offspring included 7 SS homozygotes and 7 SF heterozygotes at the Est6 locus. The absence of a second homozygous class was highly significant in all these sibships, evidence against self-fertilization by a single heterozygous parent.

Analysis of covariance found a significant difference ($p=0.010$) in F_1 survivorship among all three treatments, the 75.3% survivorship posted by the GM experiment significantly below that of the G control ($p=0.003$) but significantly greater than that of the M1 control ($p=0.0008$). Reared to maturity, one pair of F_1 progeny proved sterile in both the GM outcross and the M1 control. Production of viable F_2 by the GM progeny occurred at a median age of 10 weeks, earlier than either pure control line.

GK Experiment.

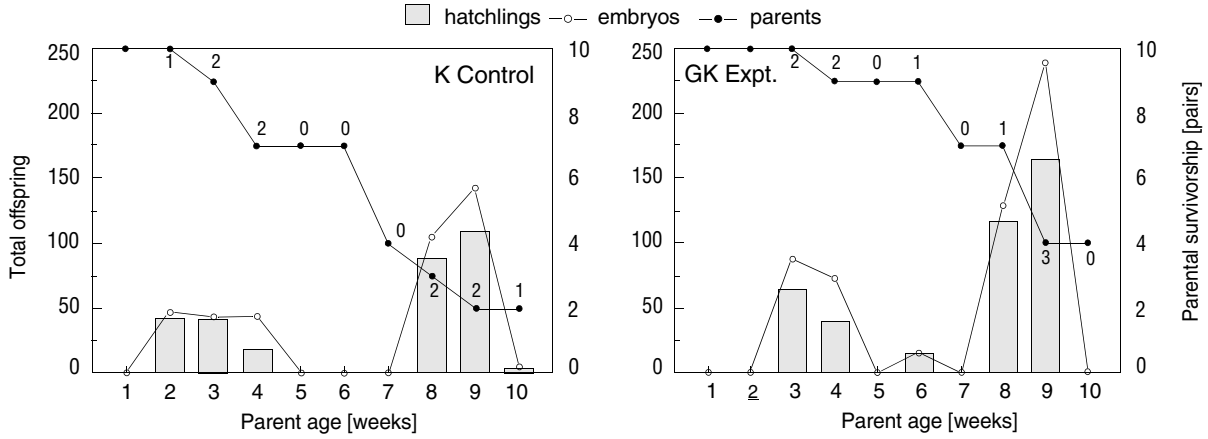

Fig. 3. Survivorship and reproduction as a function of parental age (months post hatching) for ten pairs of *Physa parkeri* (K control) and the GK outcross experiment. The corresponding *Physa gyrina* control (G) can be viewed in Figure 1. The number of reproducing pairs is given with parental survivorship (right axis).

Figure 3 compares the results of the *gyrina* x *parkeri* experiment to the *parkeri* control; the corresponding *gyrina* control again available for inspection in Figure 1. Note that both axes of Figure 1 and Figure 3 are presented in different scales, the fecundity of the GK experiment and K control being much lower than that of the G control, and their ages at first reproduction much later. One pair of K snails reproduced at week 5, two additional pairs began at week 11, and the final two pairs to reproduce did so at weeks 31 and 32, five pairs dying without issue. This was very similar to the reproductive schedule posted by the GK experiment; the five GK outcross pairs that ultimately proved fertile producing progeny first at weeks 8, 11, 12, 32 and 32.

Table 1 shows that the mean fecundity (over the entire 10 month period) for the five GK experimental pairs (83.5±43.8) was not strikingly below that of the five K control pairs (104±35.3), and that the survivorship of the putative GK hybrids was slightly better than that of the K controls. But since there was no week in which three K control pairs were reproducing, we were unable to set week 1 for an ANOVA of parental fecundity, or its corresponding ANCOVA of F_1 viability. Nor were we able to test F_1 fertility, since G control F_1 offspring were past maturity before K or GK F_1 experiments could be set up. We were, however, able to rear 56 F_1 offspring to a size analyzable by allozyme electrophoresis. The control populations proved to be fixed for alternative alleles at the Gpi locus, and our F_1 sample was unambiguously classifiable into 16 produced early in the experiment by the self-fertilization of the *gyrina* G parent, and 40 confirmed hybrids.

AV Experiment.

The results of the *ancillaria* x *aurea* trial were similar in some respects to those obtained in the GK experiment. Note the different scales on the abscissas of Figure 4, from 8-21 weeks for the AV experiment and its *aurea* control, and from 12-38 weeks for the *ancillaria* control. Only seven pairs of AV parents reproduced, their age at first reproduction (median 12.5 weeks) not significantly different from the

nine V2 control pairs (11.5 weeks). But both the AV experiment and V2 control reproduced significantly in advance of the A control, with 6 pairs first reproducing at a median age of 26 weeks.

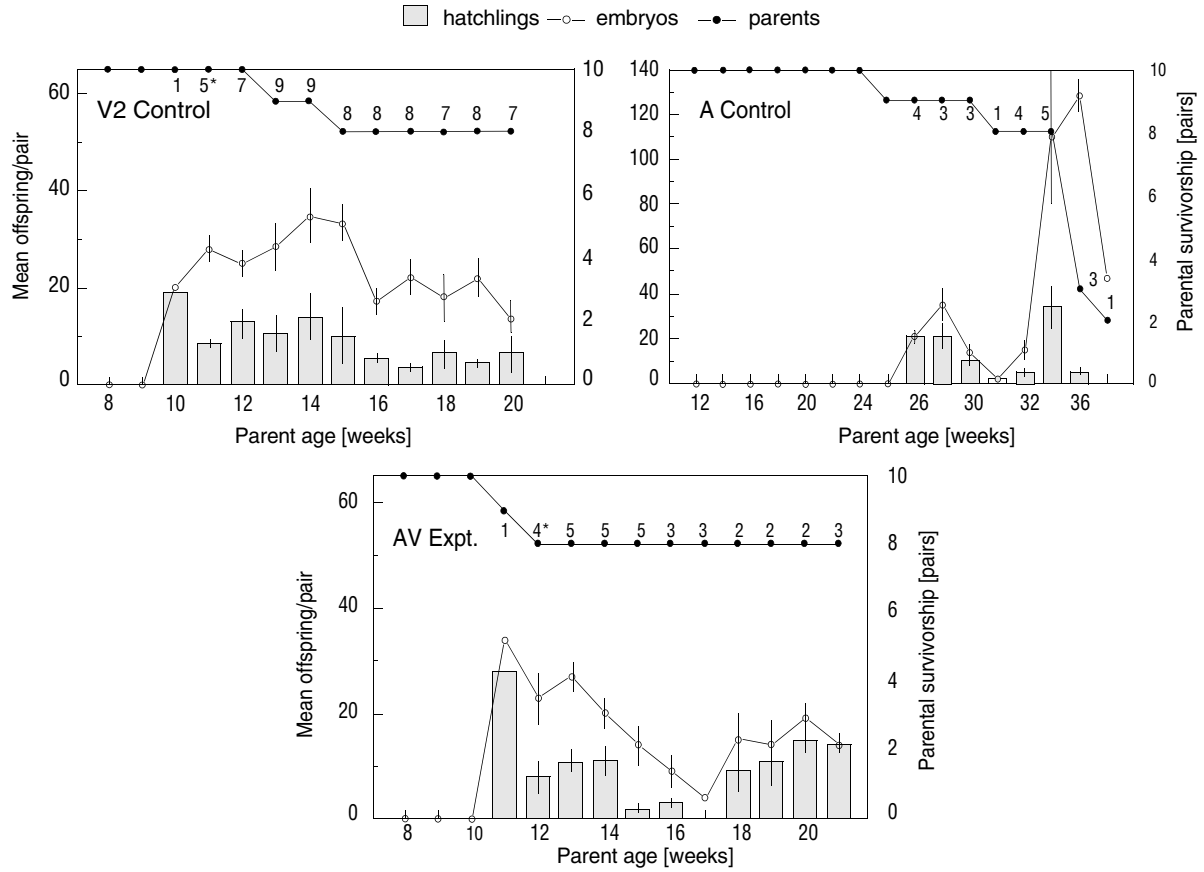

Fig. 4. Survivorship and reproduction as a function of parental age (weeks post hatching) for ten pairs of *Physa gyrina aurea* (V2 control), ten pairs of *Physa ancillaria* (A control), and the AV outcross experiment. Bars are standard errors of the mean. The number of reproducing pairs is given with parental survivorship (right axis). Asterisks* denote week 1 for analysis of variance.

After initiating reproduction at weeks 25 and 26, the A control parents showed a five-week decline in reproduction, followed by a spike in egg laying and then a striking mortality around week 36. The fecundity record of the six pairs ultimately reproducing was sparse, with many internal zeros, and hence no ANOVA of fecundity or ANCOVA of F_1 viability were possible. Nor was an assessment of F_1 fertility possible, because of the striking time lag shown by the A controls. All F_1 progeny from three AV experiments tested electrophoretically were, however, heterozygous at the Lap locus, confirming the hybrid nature of the F_1 progeny.

UM Experiment.

Figure 5 illustrates the results of the *utahensis* x *microstriata* experiment and its two corresponding controls. Some aspect of the culture environment in Tuscaloosa seems to have promoted adult survivorship; no parental mortality was recorded over any of the 30 pairs shown in Figure 5, although 3 *microstriata* pairs seem to have been sterile. There were no significant differences in age at first reproduction between the UM experiment (median=7.5 wks), the M2 control (7.5 weeks) or the U control (7 weeks). Two-way ANOVA returned a highly significant difference in fecundity ($p=0.00002$). Post hoc tests showed that the 42.2 embryos/pair/week posted by the UM experimental pairs was not significantly different from the 28.2 embryos/pair/week of the M2 control but significantly worse than the 65.6 embryos/pair/week of the U control ($p=0.0004$).

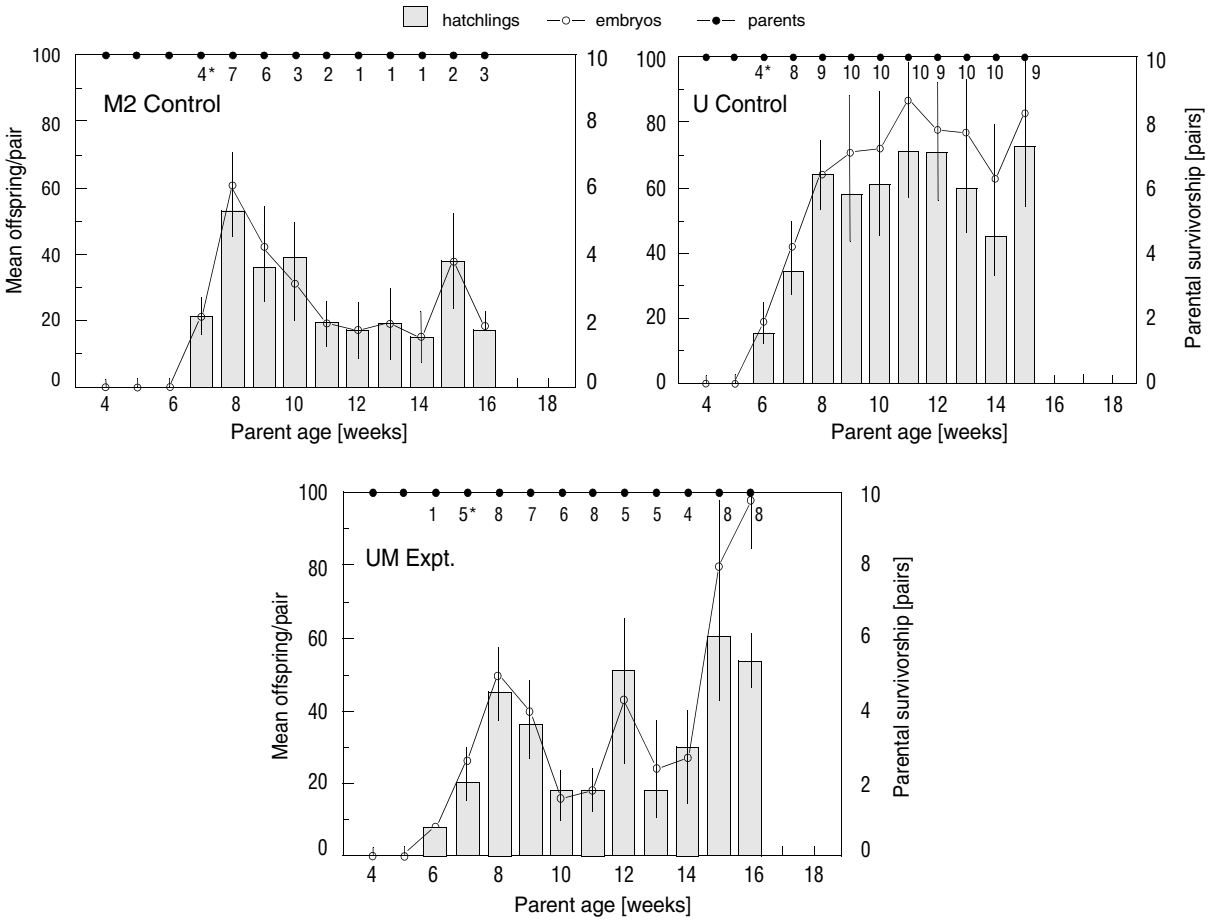

Fig. 5. Survivorship and reproduction as a function of parental age (weeks post hatching) for ten pairs of *Physa microstriata* (M2 control), ten pairs of *Physa utahensis* (U control), and the UM outcross experiment. Bars are standard errors of the mean. The number of reproducing pairs is given with parental survivorship (right axis). Asterisks* denote week 1 for analysis of variance.

Electrophoretic analysis revealed little allozyme divergence between the two control populations and little polymorphism. Thus we were unable to confirm the hybridity of large samples of F_1 offspring from several UM pairs. Twelve offspring from pair UM1 did, however, include 7 SS homozygotes and 5 FS heterozygotes at the Est6 locus, confirming the outcross. Analysis of covariance returned no significant difference among the UM experiment and its two controls in F_1 viability. Tests of F_1 fertility revealed three apparently sterile pairs among the progeny of the M2 control, one sterile pair among the U control progeny, and two sterile pairs of UM hybrids. The median age of 12 weeks at production of F_2 progeny in the remaining pairs of UM hybrids was intermediate between the ages of F_2 reproduction in the two pure line controls (Table 1).

Discussion.

The five experiments reported here uncovered striking life history diversity among populations, but no evidence of postzygotic reproductive isolation between populations.

Substantial reproductive isolation is not to be expected between topotypic *Physa gyrina gyrina* (G) and the population generally considered its subspecies, *Physa gyrina aurea* (V). Thus the results of the GV experiment served as a control at the interpopulation level for the other four experiments, all of which involved crosses that were nominally interspecific. And in fact, Figure 1 and Table 1 show that reproduction in the GV experiment was not significantly below its two corresponding incross controls by any measure. Paired GV parents did not reproduce as rapidly as pure V control pairs, but were comparable in speed to the slower G. GV parents were not as fecund as the more fertile G control, but com-

parable in fecundity to the less fertile V. And GxV F_1 hybrids matched the viability and fertility of pure-G F_1 progeny, besting pure-V F_1 progeny by a substantial margin in the former category and besting both controls in the latter.

Although involving nominally distinct species, the results of the GM, GK, AV, and UM experimental crosses were comparable in all respects to the results of the GV experiment. By every fitness criterion analyzed, Table 1 and Figures 1-5 show that outcross performance was either intermediate between the two controls, or not significantly different from at least one control. In no case did the outcross experiment perform either significantly worse or significantly better than both pure lines. Thus our data contain no evidence of reproductive isolation among any of the taxa tested here.

Our data do reflect, however, a great deal of life history divergence among these populations, apparently with a heritable basis. Although raised in a common environment, even the conspecific *P. gyrina gyrina* and *P. gyrina aurea* differed significantly in age at first reproduction, fecundity, and F_1 viability. First reproduction in control *P. ancillaria* was delayed to such an extent behind control *P. gyrina aurea* that pure-*aurea* and hybrid F_1 offspring were mature before pure-*ancillaria* F_1 offspring were born. A similar situation pertained in the *gyrina* x *parkeri* experiment, where reproduction in the *parkeri* control lines lagged months behind the *gyrina* control, ultimately precluding any assessment of the fertility of their hybrids. Thus we were unable to rule out hybrid sterility in our *gyrina* x *ancillaria* and *gyrina* x *parkeri* experiments. Mitochondrial sequence data suggest that *parkeri* and *ancillaria* may belong to a colder or more northern *Physella* group, while our other four nominal taxa belong to a warmer-adapted group (WETHINGTON & GURALNICK in press, WETHINGTON & LYDEARD in review).

The great diversity in life history pattern displayed by our control populations is unsurprising, given the diversity of the habitats from which they were collected. Our populations were spread across 7 degrees of latitude and 36 degrees of longitude, three in lotic environments and three in lentic. The hot springs outflow from which our sample of *P. gyrina aurea* was collected is only about 2 meters wide, with moderate flow over sand/cobble bottom. The Boyer River at the point we collected our topotypic *P. gyrina* is approximately 15-20 meters wide, with a mud bottom and negligible current. We sampled *P. ancillaria* from quiet pools at the margins of the Delaware River, 100-200 m wide with a strong current and rocky bottom. Our sample of *P. parkeri* was collected from a rocky, exposed shore of the 1500 hectare mesotrophic Douglas Lake, Michigan. Our samples of *P. microstriata* and *P. utahensis* were collected from mixed substrates on the protected shores of strikingly different lakes: the 1000 hectare Fish Lake (an oligotrophic body of soft water) and the 39000 hectare Utah Lake (a hypereutrophic body of very hard water).

The single attribute that unites these environments is that all six are relatively predictable. Physid populations of the subgenus *Physella* are not characteristic of disturbed or temporary habitats, unlike populations of the other major physid subgenus, *Costatella*. The mean fecundities shown in Table 1 generally range around 20 embryos/pair/week, and are often much lower. These figures are strikingly below the levels of fecundity typically attained by *Costatella*, which reproduce earlier and average 40-60 embryos/pair/week under identical conditions (DILLON & al. 2002; DILLON & al., in press). We suggest that *Costatella* populations such as *P. acuta* may show R-selected life history adaptation (in the sense of DILLON 2000:131-135), while *P. gyrina* and *Physella* populations generally may be more undifferentiated in this regard.

Our results are consistent with the hypothesis that *P. ancillaria* (SAY 1825), *P. parkeri* (CURRIER 1881), *P. utahensis* (CLENCH 1925) and *P. microstriata* (CHAMBERLAIN & BERRY 1930) are all synonyms of *Physa gyrina* (SAY 1821). The life history divergence displayed by these populations is doubtless adaptive to the diverse environments they inhabit, but does not seem to be accompanied by postzygotic reproductive isolation. The difficulties we experienced culturing *P. ancillaria* and *P. parkeri* in particular using our standard techniques introduced an element of ambiguity into these findings, however. Our ongoing surveys of allozyme and sequence divergence among wild populations of a broad sample of physid taxa will cast further light on specific synonymy within this diverse group.

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