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Fitness benefits of diverse offspring in pygmy grasshoppers

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ABSTRACT

Question: Do females obtain fitness benefits from an increase in offspring diversity?

Hypotheses: Polyandry increases offspring diversity within a clutch. Increased offspring diversity will reduce competition among siblings (manifested as increased mean survival in more diverse families) and the probability that all offspring might be ill-suited to future conditions (manifested as lower variance in survival in diverse families).

Organisms: Pygmy grasshoppers, *Tetrix subulata* and *Tetrix undulata*, that are polymorphic for colour pattern.

Field site: South-central Sweden.

Methods: We varied the number of mates provided to colour polymorphic pygmy grasshoppers. We reared families in either warm or cold conditions using a split-brood design.

Conclusions: The colour morph diversity of broods increased with the number of experimentally provided mates. Colour morphs represent alternative strategies that differ in body size, physiology, behaviour, and life history. Survival increased with increasing sibling diversity, supporting the hypothesis that different morphs avoid competition by using different subsets of available resources. Homogeneous families (in which all siblings belong to the same or only a few colour morphs) varied more in survival than did families with diverse siblings, supporting the hypothesis that morphs vary in their ability to cope with and utilize different resources.

Keywords: colour polymorphism, offspring diversification, polyandry, selective free-rider.

INTRODUCTION

It has been proposed that females that mate with multiple males may obtain fitness benefits mediated via increased diversity among individual offspring, assuming that multiple mating results in clutches with mixed parentage (Ridley, 1993; Yasui, 1998, 2001; Zeh and Zeh, 2001; Foerster *et al.*, 2003). Polyandrous females may avoid having all their offspring fathered by a low-quality or genetically incompatible male, but such within-generation bet-hedging for good genes is generally believed to be unimportant, except in very small populations where it may help avoid the detrimental effects of demographic stochasticity (Dempster, 1955; Yasui, 1998, 2001;

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Hopper *et al.*, 2003; Sarhan and Kokko, 2007). Producing diversified offspring could, however, entail fitness benefits in the context of between-generation bet-hedging. In environments that fluctuate unpredictably, a behavioural strategy (such as polyandry) that results in variable phenotypic expression among progeny may reduce the probability that all offspring will become ill-suited to future conditions and increase geometric mean fitness in changing environments (Gillespie, 1974; Slatkin, 1974; Seger and Brockmann, 1987; Philippi and Seger, 1989; Yasui, 1998, 2001; Jennions and Petrie, 2000; Fox and Rauter, 2003; Hopper *et al.*, 2003). A polyandrous strategy that produces diverse offspring may thus experience a reduced variance in fitness among years with different conditions, or across environments within years, compared with a monandrous strategy that produces more uniform offspring.

Another potential benefit (of the non-bet-hedging type) of increased phenotypic diversity among individual offspring is that it may reduce competition among siblings if different phenotypes utilize different subsets of available resources (Van Valen, 1965; Moran, 1992; Yasui, 1998; Ahnesjö and Forsman, 2006). Reduced competition among diversified offspring is especially likely if colour morphs represent alternative ecological strategies with different co-adapted gene complexes and utilize, on average, different resources (Forsman *et al.*, in press). A female may potentially increase offspring diversity either by mating with multiple males or by mating disassortatively – that is, with a male that is phenotypically and genetically different from herself. However, fitness benefits mediated via increased offspring variability has received relatively little attention (Yasui, 1998, 2001; Baer and Schmid-Hempel, 1999; Zeh and Zeh, 2001).

Here we report on a study of colour polymorphic pygmy grasshoppers (Orthoptera: Tetrigidae) performed to test for fitness benefits of offspring diversification. Pygmy grasshoppers are small (up to 15 mm body length, 0.07 g on average), diurnal, ground-dwelling insects that mostly inhabit tropical rainforests but also temperate and even arctic regions of Europe, Asia, and America. They locally attain high population densities, particularly in recently disturbed areas such as clear-cuttings (Rowell, 1971; Forsman and Appelqvist, 1999). They exhibit a multitude of genetically influenced (i.e. heritable) colour morphs within populations; individuals may vary from black, through yellowish-brown, to light grey, with some being monochrome and others having distinct patterns (Nabours, 1929; Fisher, 1930, 1939; Forsman, 1999). In some tetrigids, colour morphs represent alternative ecological strategies that differ in body size, clutch size, and inter-clutch interval, body temperature preferences and sensitivity of performance to temperature change, thermoregulatory behaviour, anti-predator behaviour, and microhabitat selection (Forsman, 1999, 2000; Forsman and Appelqvist, 1999; Forsman *et al.*, 2002; Ahnesjö and Forsman, 2003, 2006, and references therein). Some of these associations are based on phenotypic correlations only, but available evidence indicates a genetic contribution to the variation among morphs in thermoregulatory behaviour (Forsman *et al.*, 2002) and body size (Ahnesjö and Forsman, 2003).

The pygmy grasshopper populations investigated here inhabited burnt, clear-cut fields in south-central Sweden. In recently burnt areas that undergo changes in vegetation, background colour, and community composition, populations may increase in numbers and thrive for a few years before declining again to low background levels following the recurrence of vegetation (Holst, 1986; Forsman and Appelqvist, 1999). The frequencies of colour morphs vary among populations and between years within populations (Rowell, 1971; Forsman and Appelqvist, 1999; Karlsson *et al.*, in press), which suggests that relative fitness of alternative morphs may change across conditions.

We have previously shown that female pygmy grasshoppers mate indiscriminately with regard to colour morph and that they frequently mate with multiple males, both in natural populations and under laboratory conditions (Caesar *et al.*, 2007). Here, we experimentally mate females to different numbers and diversity of males (no, one, two similar or two different colour morph males) to test for effects of polyandry on variability of offspring, and manipulate variation in offspring diversity among families to test for associations with survival. In addition, we perform a split-brood experiment to test for effects of among-sibling diversity on survival of families that are reared under two different thermal environments. Because pygmy grasshopper colour morphs represent ecological strategies that utilize different resources, we expect more diverse families to be better able to cope with variable environments and to suffer less from competition, and hence experience superior survival.

METHODS

Study animals and mating experiment protocol

We collected adult *T. subulata* grasshoppers on two occasions in spring 2004 (5 May and 18 May) from a natural population inhabiting a 3-year-old, burnt, clear-cut field of approximately 10 ha in south-central Sweden, and housed females in separate plastic containers during transportation to the laboratory. Within 24 h of capture, we transferred females to individual plastic containers measuring 150 × 85 × 210 mm maintained in the laboratory at 28 ± 1°C, 75% relative humidity, and a 12 h/12 h light/dark cycle. Four fluorescent strip lights (Philips Master TL 28W/830 HE) mounted in the ceiling were used as a complement to the natural light. We used two humidifiers (Aquastar-NT, Burg BH-850 E, Honeywell Inc.) to maintain air humidity at a stable level. Each cage contained a small aluminium cup (25 mm in diameter) of moist cotton for drinking and an identical cup filled with a 1 : 1 mixture of moist peat and soil as food and oviposition media (Forsman *et al.*, 2002; Caesar *et al.*, 2007). A slice of fresh potato was provided as food.

We randomly assigned females belonging to different colour morphs to one of four mating treatments, subject to the constraint that colour morphs should be evenly distributed among treatments:

- *no male* ($n = 45$ replicates): females were kept alone in plastic cages with no male to control for and quantify background noise due to the possible influence of mating performed in the field prior to capture;
- *one male* ($n = 37$ replicates): females were kept together with one male;
- *two similar males* ($n = 33$ replicates): females were kept together with two different males, both of which belonged to the same colour morph;
- *two dissimilar males* ($n = 37$ replicates): females were kept together with two males that belonged to two different colour morphs.

In some cases, the female belonged to the same colour morph as one or both males; in other cases, the female did not resemble the male(s). We did not observe matings in this study but we do have evidence from a previous experiment (Caesar *et al.*, 2007) that the majority of females mate polyandrously when given the opportunity. For instance, 75% of females experimentally presented two different males mated with both males within 48 h, and all females mated with at least one of the two males. Given that females were kept together with the male(s) during the entire experiment in the present study, we are quite confident that those females that were presented with two males mated with both of them. The fact that some females may have mated in the field prior to the experiment may be manifested as background noise but is unlikely to create a systematic bias among treatments (see also Discussion). To our knowledge, it has not been investigated if last-male sperm precedence exists in pygmy grasshoppers, but that would tend to reduce expected differences between our experimental mating treatments.

We examined the cages for egg pods every third to fourth day. Egg pods were placed on a piece of moist cotton inside a plastic Petri-dish (80 mm in diameter) for incubation under the same temperature conditions as where they were laid. We checked egg pods every second day, and recorded hatching date and number of hatched young. Because it is not possible to reliably determine colour morphs of newly hatched nymphs, we postponed classification and quantification of within-family diversity until a later date (see below). Several days after the last egg had hatched, we examined egg pods under a stereo-microscope and recorded the number of eggs that had failed to hatch. Upon their second oviposition females were freeze-killed and stored at −20°C for subsequent analysis. Some of the 152 females included in the experiment died at an early stage. In addition, some females failed to oviposit; at the end of the experiment, three of the surviving females had not produced a single clutch and six females had laid only one clutch. Sample sizes may therefore deviate from the initial number of replicates (see Table 1).

Hatchlings from first clutches were used as source for propagules in an experiment designed to test for an effect of genetic diversity on colonization success. We therefore have no data on offspring post-hatching for first clutches. However, we reared hatchlings from second clutches by family in separate, white 10-litre plastic buckets containing a peat–soil mixture placed out of doors [for rearing conditions, see Forsman *et al.* (2002) and Ahnesjö and Forsman (2003)]. In late summer (18 August), we thoroughly examined each bucket,

Table 1. Reproductive success (mean ± standard deviation) of, and survival of offspring born to, female pygmy grasshoppers maintained alone or mated to one male, two males belonging to the same colour morph or two males belonging to different colour morphs

	Male treatment			
Variable	No male	One male	Two similar males	Two dissimilar males
Number of replicates	45	37	33	37
Data for first clutch, *N*	*37*	*32*	*26*	*34*
Number of eggs	16.02 ± 9.46	17.65 ± 9.55	15.58 ± 9.59	18.35 ± 8.50
Proportion of eggs that hatched	0.60 ± 0.41	0.62 ± 0.34	0.65 ± 0.34	0.78 ± 0.31
Proportion of clutches that failed (± 95%CI)	0.22 (0.136)	0.12 (0.115)	0.07 (0.100)	0.03 (0.059)
Data for second clutch, *N*	*28*	*29*	*26*	*27*
Number of eggs	7.79 ± 2.32	8.28 ± 3.59	8.31 ± 3.77	8.59 ± 4.54
Proportion of eggs that hatched	0.54 ± 0.39	0.62 ± 0.44	0.61 ± 0.36	0.57 ± 0.41
Proportion of clutches that failed (± 95%CI)	0.15 (0.135)	0.28 (0.167)	0.12 (0.127)	0.26 (0.169)
Proportion of offspring surviving	0.47 ± 0.3	0.46 ± 0.39	0.48 ± 0.37	0.57 ± 0.33

counted and classified grasshoppers by developmental stage (nymph or adult), colour morph, and sex. We thereafter placed males and females in separate cages outdoors for continued growth and over-winter survival.

For each female we recorded the number of eggs in the first and second clutch, the proportion of eggs failing to hatch in each clutch, and offspring survival, measured as the proportion of hatchlings (from second clutches only) that were alive in late summer (18 August). As a measure of heterogeneity (with respect to colour morph) among offspring within a clutch, we computed for each clutch the number of colour morphs. Because a small family cannot harbour as many different colour morphs as a large family, any comparison of diversity among mating treatments may be confounded by differences in family size. When testing for differences in offspring diversity between treatments, we therefore include number of individuals within each family as a covariate and compute least squares means for the different treatments. We used logit models suitable for modelling and analysing binary data in the form of proportions (Collett, 1991) to test for variation among mating treatments in the proportion of hatched eggs and failed clutches. We computed maximum likelihood estimates of the parameter values using the procedure CATMOD (for proportion failed clutches) or GENMOD (SAS Institute Inc., 2004). When necessary, we used a common variance inflation factor for all observations (computed as the square root of the Pearson's chi-square divided by the degrees of freedom) to account for problems associated with over-dispersion. Our analyses of effects of mating treatment and within-family diversity on offspring survival are described below.

Quantifying natural offspring diversity

To quantify among-sibling diversity in clutches produced by females mated under natural conditions, we use information on offspring that were raised in captivity and born to *Tetrix subulata* females that were collected as adults (and assumed to have mated prior to capture) on 25 May 2005 from a natural population and brought to the laboratory where they were housed individually (without access to males) for egg-laying in 10-litre plastic buckets maintained out-of-doors during summer. On 5 September, the buckets were examined for offspring, which were counted (mean number of siblings per family = 9.7, standard deviation = 7.12, range = 1–29 individuals, $n = 46$ families) and classified by colour morph. We quantify natural offspring diversity also in *Tetrix undulata*, as described below.

Testing for effects of among-offspring diversity on survival

To test for effects of among-sibling diversity on survival, we use information on offspring that were raised in captivity and born to *Tetrix undulata* females that were collected as adults (and assumed to have mated prior to capture) on two occasions (26 April and 11 May) in spring 2000 from a natural population. We brought females to the laboratory where they were housed individually (without access to males) for egg-laying. [For information on source population and rearing conditions, see Forsman *et al.* (2002) and Ahnesjö and Forsman (2003).] A total of 75 wild-caught females produced clutches that yielded at least seven live hatchlings (mean number of hatchlings = 20.4, standard deviation = 7.09, range = 7–32).

Upon hatching, we split each litter into two halves and placed each half in a separate cage such that offspring from each female were reared in both cold and warm temperature regimes in a greenhouse using a split-brood design (Forsman *et al.*, 2002; Ahnesjö and Forsman, 2003). As explained above, it is not possible to reliably determine the colour morph of newly hatched nymphs. Buckets were therefore examined for surviving grasshoppers, which were counted and classified by colour morph both on 9–15 August and 5–8 September. We tested if offspring variability measured as number of colour morphs at first census was a reliable predictor of survival from thereon until the end of the experiment – that is, between the first and second census. Because offspring from each female were reared under two different environments, survival was analysed using a repeated-measures approach, regarding survival of siblings reared under cold and warm temperatures as a repeated measure using logit-model analysis implemented with procedure GENMOD (SAS, 2004). Maternal colour morph was included as an explanatory variable. To control statistically for a possible confounding effect on survival of number of individuals initially released in each bucket (Ahnesjö and Forsman, 2003), we also included number of hatchlings as a continuous explanatory variable in the model. We started with a saturated most complex model and excluded non-significant explanatory variables through an iterative fitting process. The Wald statistic was used to assess the statistical significance of explanatory variables included in the model (Collett, 1991). A common variance inflation factor for all observations (computed as the square root of the Pearson's chi-square divided by the degrees of freedom) was used to account for problems associated with over-dispersion.

RESULTS

Clutch size and hatching success

We found no effect of mating treatment (no, one, two similar or two different colour morph males) on clutch size or on the change in number of eggs between first and second clutches (Table 1). Number of eggs declined from first to second clutch [multivariate analysis of variance for repeated measures as described with examples in O'Brian and Kaiser (1985), treating observations for the first and second clutch produced by a female as a repeated measure; effect of clutch number: Wilks' $\lambda = 0.24$, $F_{1,101} = 314.5$, $P < 0.0001$], but there was no difference in overall clutch size between females presented with different number and diversity (0, 1, 2 similar or 2 different) of males ($F_{3,101} = 0.10$, $P = 0.96$). Mating treatments also did not differ with regard to the change in number of eggs between the first and second clutch (effect of clutch × treatment interaction: Wilks' $\lambda = 0.999$, $F_{3,101} = 0.03$, $P = 0.99$) (Table 1). The results were unchanged when the data were re-analysed after including also those females that did not lay a second clutch (i.e. assigning their second clutch size a value of zero).

Our data for *T. subulata* females experimentally mated to no, one, two similar or two different colour morph males uncovered no apparent effects of mating treatment on viability of eggs and offspring. The direction of differences in hatching rate of eggs among first clutches ($n = 129$) was consistent with the hypothesis that an increasing number and diversity of sires positively influences hatching success (Table 1) but the variation among treatments was not significant (logit-model analysis using GENMOD; effect of mating treatment: $\chi^2 = 4.01$, d.f. = 3, $P = 0.26$). The situation was similar with respect to the proportion of failed clutches (i.e. clutches in which no egg hatched, logit-model analysis using CATMOD; effect of mating treatment: $\chi^2 = 5.59$, d.f. = 3, $P = 0.13$) (Table 1). Among second clutches, there also was no significant effect of mating treatment on hatching rate (logit-model analysis using GENMOD; effect of mating treatment: $\chi^2 = 0.98$, d.f. = 3, $P = 0.81$), or on the proportion failed clutches ($\chi^2 = 3.23$, d.f. = 3, $P = 0.36$) (Table 1).

Variability among offspring

Data on *T. subulata* hatchlings from second clutches that were alive at the end of summer (mean = 4.1, standard deviation = 3.16, range = 1–15 individuals, $n = 69$ clutches) uncovered considerable variability among siblings with regard to colour morph (median number of morphs within a clutch = 2, mean = 1.97, standard deviation = 1.06, range = 1–5 colour morphs). The diversity (as estimated by number of different colour morphs) increased with number and diversity of experimentally provided mates and was highest for offspring born to females mated to two males that belonged to different colour morphs (analysis of covariance including mating treatment as a factor and number of surviving siblings as covariate to statistically control for differences in family size; effect of number of siblings: $F_{1,64} = 37.97$, $P < 0.0001$; effect of mating treatment: $F_{3,64} = 3.86$, $P = 0.013$) (Fig. 1). Comparisons of least squares means between treatments revealed that females mated to one male produced less diverse offspring than did females mated to two similar males ($P = 0.024$) and females mated to two different males ($P = 0.0035$). The difference in offspring diversity between females mated to two similar males and two different males was not significant ($P = 0.51$). The diversity of offspring born to females in the no-male control group differed significantly ($P = 0.025$) only from that of females in the treatment with two different males. The effect of mating treatment on offspring diversity remained significant also when data for the group of females included in the no male treatment were omitted from the analysis ($F_{2,48} = 4.61$, $P = 0.015$).

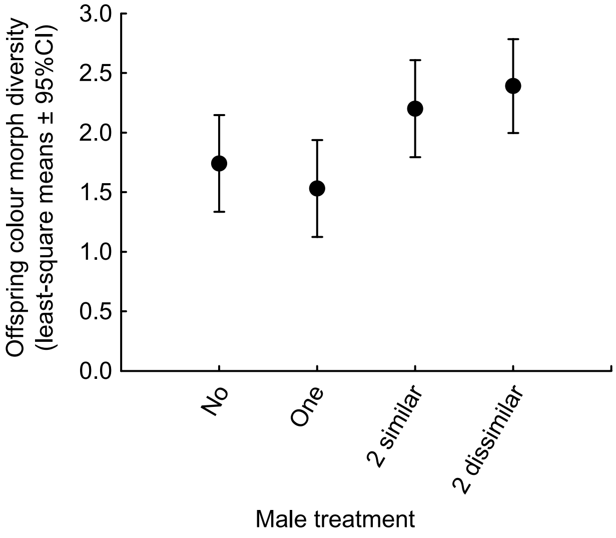

Fig. 1. Effect of mating treatment on offspring diversity. Colour morph diversity among offspring born to female *Tetrix subulata* experimentally mated to no, one, two similar or two dissimilar males. Figures show least squares means ± 95% confidence interval, as obtained from an analysis of covariance with number of siblings as a covariate (see text for details).

Colour morph diversity in 'natural' clutches and parent–offspring resemblance

The within-clutch variability in morph diversity reported above was not merely a consequence of artificial conditions. Data based on offspring born to females mated in the field prior to capture (Forsman *et al.*, 2002; Ahnesjö and Forsman, 2003) also uncover considerable diversity among siblings with regard to colour morph in both *Tetrix undulata* (median = 3, mean = 2.9, standard deviation = 1.2, range = 1–7 color morphs, $n = 75$ clutches) and *T. subulata* (median = 2.5, mean = 2.6, standard deviation = 1.3, range = 1–5 color morphs, $n = 46$ clutches) (Fig. 2). Parent–offspring resemblance of colour pattern (measured as the percentage of offspring within a clutch that belong to the same colour morph as their mother) was moderate in both species (*T. undulata*: mean = 36%, standard error = 3.4%, range = 0–100%, $n = 75$; *T. subulata*: mean = 32%, standard error = 4.6%, range = 0–100%, $n = 46$).

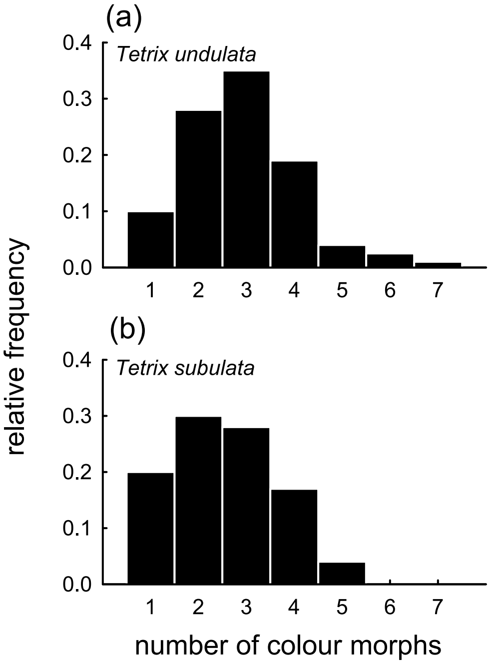

Fig. 2. Offspring diversity in natural clutches. Relative frequency distribution of clutches as a function of number of colour morphs available among siblings within the same clutch. (a) Data for *Tetrix undulata* families (mean number of siblings per family = 15.1, standard deviation = 6.64, range = 1–28 individuals, $n = 75$ families) born to wild-caught females (mated in the field prior to capture) and raised in captivity in either cold or warm conditions, using a split-brood design (for details, see Forsman *et al.*, 2002; Ahnesjö and Forsman, 2003). (b) Data for *Tetrix subulata* families (mean number of siblings per family = 9.7, standard deviation = 7.12, range = 1–29 individuals, $n = 46$ families) born to wild-caught females (mated in the field prior to capture) and raised in captivity.

Variability among offspring promotes survival

We found evidence that females may increase their fitness by producing more variable offspring. Our mating experiment showed that survival of nymphs was positively associated with colour morph diversity among siblings (logit-model analysis using GENMOD, effect of number of colour morphs: $\chi^2 = 17.02$, d.f. = 1, $P < 0.0001$) (Fig. 3a) but not influenced by mating treatment *per se* ($\chi^2 = 4.29$, d.f. = 3, $P = 0.23$) (Table 1), other than indirectly via the positive effect mediated by offspring diversity. Survival of nymphs also was not affected by number of released hatchlings ($\chi^2 = 0.02$, d.f. = 1, $P = 0.89$) or maternal colour morph ($\chi^2 = 7.34$, d.f. = 3, $P = 0.07$). Although these findings are consistent with the hypothesis that survival of nymphs is greater in more diverse families, we cannot disentangle cause from effect in this data set, because offspring diversity was recorded at the end of the experiment. However, the results from our split-brood experiment reported below do provide unequivocal evidence.

Among hatchlings produced by wild-caught *T. undulata* females that we experimentally reared under warm and cold conditions using a split-brood design (Forsman *et al.*, 2002; Ahnesjö and Forsman, 2003), we found direct evidence that survival increases among families with increasing within-family diversity. The number of colour morphs within the clutch in mid-August reliably predicted survival from thereon until mid-September [repeated-measures logit-model analysis using GENMOD with treatment (warm or cold) as a repeated measure, maternal colour morph as a factor, and number of hatchlings released in the cage as a covariate; effect of number of colour morphs: $\chi^2 = 12.13$, d.f. = 1, $P < 0.0005$) (Fig. 3b). Survival also was negatively affected by increasing number of hatchlings released ($\chi^2 = 17.11$, d.f. = 1, $P < 0.001$) and varied depending on maternal colour morph ($\chi^2 = 884.02$, d.f. = 10, $P < 0.0001$) but was not influenced by rearing temperature ($\chi^2 = 0.64$, d.f. = 1, $P = 0.42$). The effect of number of colour morphs also did not differ between rearing temperatures (as evidenced by a non-significant interaction: $\chi^2 = 0.01$, d.f. = 1, $P = 0.94$). Diversity among siblings in mid-August was associated also with survival of nymphs from the stage of hatching until mid-September (effect of number of colour morphs: $\chi^2 = 8.94$, d.f. = 1, $P < 0.01$) (Fig. 3c). The effects of the remaining variables on survival from hatching until mid-September (not shown) were qualitatively similar to those reported for the restricted time period.

As discussed in the Introduction, variance in fitness is central to bet-hedging. We find that the variability among families in offspring survival (estimated across families as the standard deviation of the mean proportion surviving siblings, as shown in Fig. 3) appears to decrease with increasing within-family diversity (as evidenced by the regression analysis of standard deviation against number of colour morphs, August to September: $F_{1,3} = 8.36$, $R^2 = 0.74$, $P = 0.063$; 95% confidence interval of regression slope as obtained using bootstrap = −0.095 to 0.00063; June to September: $F_{1,3} = 16.00$, $R^2 = 0.84$, $P = 0.028$, bootstrapped 95% CI = −0.066 to −0.039). This reduced variability in survival with increasing offspring diversity is not a spurious result caused by differences in sample size (standard deviation was not correlated with the number of families in each category of number of colour morphs, August to September: $r = -0.14$, $n = 5$, $P = 0.82$; June to September: $r = 0.02$, $n = 5$, $P = 0.97$).

Finally, the variability in survival within families but across the two thermal environments decreased with increasing diversity among siblings (absolute values of mean

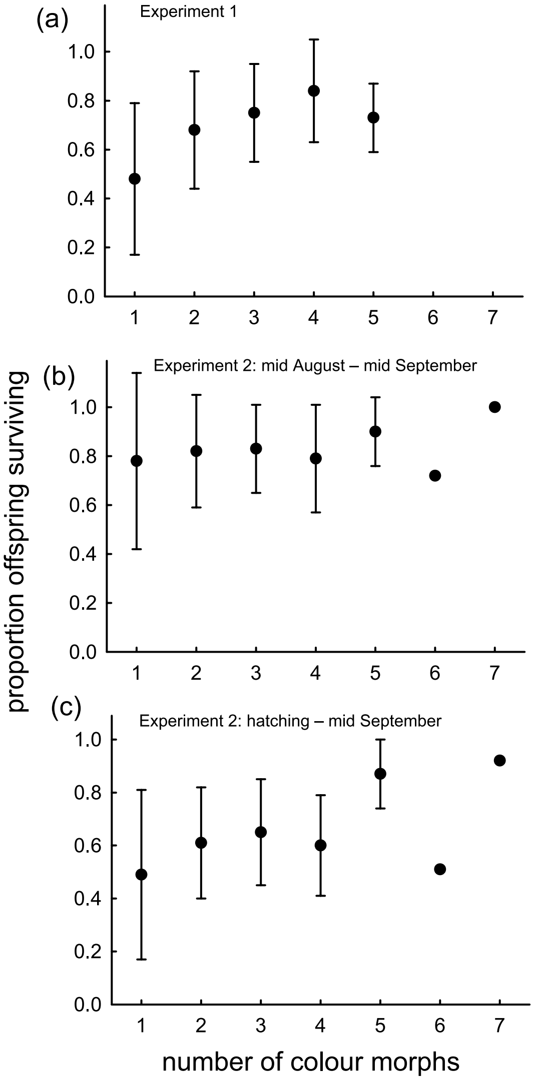

Fig. 3. Survival (mean ± standard deviation) of offspring in relation to within-family colour morph diversity. (a) Data for *Tetrix subulata* that were born to females that had been experimentally mated to no, one, two similar or two dissimilar males. (b, c) Data for *Tetrix undulata* that were born to wild-caught females and reared in captivity in either cold or warm conditions, using a split-brood design (for details, see Forsman *et al.*, 2002; Ahnesjö and Forsman, 2003). Proportion of offspring surviving from mid-August to mid-September (b) and from June to mid-September (c) as a function of among-offspring colour morph diversity measured as the number of colour morphs available in mid-August.

between-treatment difference in proportion offspring that survived from mid-August to mid-September were negatively associated with number of colour morphs in the family, $r = -0.79$, $n = 7$, $P = 0.032$).

DISCUSSION

Our results suggest that increased variability among offspring may translate into fitness benefits (Fig. 3) for females. The positive effects of producing more diverse offspring may be two-fold: reduced competition among siblings (manifested as enhanced overall survival in more diverse families), and reduced probability that all offspring be ill-suited to existing conditions (manifested as a lower variance in survival among more diverse families).

We assigned females randomly to mating treatments, such that any pre-experimental matings would be manifested as background noise (increased variance within treatments) rather than create a systematic bias among treatments. Even if some females were already mated prior to the experiment, the females in our two-male treatments will on average have mated to more males than females in our one-male treatment. This is supported by the finding that our pygmy grasshopper females experimentally mated to multiple males produce more diversified offspring (Fig. 1). The latter finding suggests that polyandry results in mixed paternities (i.e. half-siblings) and conforms to studies of other species of pygmy grasshopper where polyandrous females produced clutches sired by up to seven different males (Nabours, 1929). Because there is considerable diversity (with up to seven different colour morphs) also among siblings born to *T. subulata* and *T. undulata* females mated in the wild prior to capture (Fig. 2), we are confident that this intra-clutch diversity is not simply caused by artificial conditions, but reflects instead a natural situation.

It is not surprising that *T. subulata* females captured in nature produced families that consisted of on average 2.6 morphs, while females that were also captured in nature but subsequently exposed to different mating treatments in captivity produced families with on average less than 2.6 morphs. The diversity estimate for offspring to wild-caught females is based on more than twice as many offspring (9.7 vs. 4.1) as that for experimentally mated females. In addition, the number of colour morphs increases with family size. When the difference in average family size is accounted for, experimentally mated females produce clutches characterized by higher (not lower) diversity with regard to number of colour morphs. This reinforces the conclusion that the matings in captivity increased the level of diversity among offspring beyond the expected level of background noise.

Our split-brood experiment shows that survival of offspring from mid-August until mid-September is higher in families characterized by high among-sibling diversity than in more homogeneous families. We assessed survival after the scoring of the morphs, and disentangling cause from effect is therefore not an issue in this data set. We cannot identify the mechanism behind the enhanced survival of more diverse families. It may reflect a lower susceptibility to disease transfer (Baer and Schmid-Hempel, 1999) or, more likely, reduced competition between half siblings (Ridley, 1993) because different colour morphs utilize different resources (Van Valen, 1965; Moran, 1992; Yasui, 1998; Leimar, 2005; Ahnesjö and Forsman, 2006; Forsman *et al.*, in press). The interpretation that the positive influence of diversity on offspring survival is mediated by reduced competition between individuals belonging to different ecomorphs is supported by the finding that survival was density dependent (survival decreased with increasing number of released hatchlings). That no such effect of crowding was evident on survival of nymphs in our mating experiment may simply reflect the smaller average family size, as compared to our split-brood experiment (approximately 5 vs. 10 hatchlings released per bucket). Available mark–recapture data and behavioural observations of free-ranging adult individuals suggest that pygmy grasshoppers are sedentary animals that perform short-distance movements within the order of a few metres per day (Forsman and Appelqvist, 1999;

Ahnesjö and Forsman, 2006). It is therefore plausible that offspring stay together and interact locally in nature during nymphal development, such that our results are applicable also to a natural situation.

An interesting finding in our study, with respect to bet-hedging theory, is that the variance in offspring survival is higher among homogeneous families (in which all siblings belong to the same or only a few colour morphs) than among families with diverse siblings. If colour morphs vary in their ability to cope with different environmental conditions, then all of the offspring in a homogeneous family are expected to do equally badly, intermediate or well. This will result in low variation in success among individual offspring within homogeneous families, but large variation among families. However, in a diverse family, where siblings belong to several different morphs, some offspring are expected to do good whereas others are expected to be less fortunate. This will result in large variation in success among individual offspring within diverse families and small variation among diverse families. The above patterns are evident in our data (Fig. 3). Our results also support the prediction from bet-hedging theory of lower variability in offspring survival across environments (warm or cold) for females that produced more diverse offspring.

Polyandry in pygmy grasshoppers may be considered as a diversified bet-hedging strategy (Seger and Brockmann, 1987; Philippi and Seger, 1989), in the sense that the reduced variance in success among females is achieved by means of an increased phenotypic and fitness diversification among individual offspring. A diversified bet-hedging strategy must be realized by a variable phenotypic expression of a single genotype, and genetic polymorphisms therefore should not be considered bet-hedging (Seger and Brockmann, 1987; Hopper *et al.*, 2003; Leimar, 2005). While available evidence suggests that the existence within populations of several different colour morphs reflects an underlying genetic polymorphism, it is plausible that the decision to mate with one or several males may be influenced by a single gene. We propose, therefore, that polyandry 'exploits' the polymorphism as a means of creating phenotypic diversification, and so it might constitute a genuine bet-hedging strategy [confer the 'lottery' metaphor for sex and recombination in Seger and Brockmann (1987)]. However, an important unanswered question is whether the diversification of offspring caused by polyandry results in increased geometric mean fitness over generations. A female need not necessarily be polyandrous to produce more variable progeny. Female pygmy grasshoppers may increase diversity among their offspring by mating with a male that belongs to a colour morph different from their own (S. Caesar and A. Forsman, unpublished data). However, available evidence suggests that they mate indiscriminately with regard to colour morph, perhaps because they are unaware of their own morph or unable to discriminate among male morphs (Caesar *et al.*, 2007).

Bet-hedging is often described as a strategy that reduces variance in individual fitness at some cost to expected (arithmetic) mean fitness (Seger and Brockmann, 1987; Philippi and Seger, 1989; Hopper *et al.*, 2003). Our findings suggest that females that produce more diverse offspring enjoy higher mean fitness as well as lower variance in fitness. We propose as an explanation for this result that the bet-hedging benefit of producing diverse offspring, manifested as reduced variance in survival, co-occurs with another benefit (of the non-bet-hedging type), namely reduced competition among siblings, manifested as increased mean overall survival.

Theory posits that discrete polymorphisms can be maintained only under a restricted set of conditions, with heterozygote advantage, negative frequency dependence or opposing selection in different sexes or environments considered to be the most powerful mechanisms (Haldane, 1962; Ford, 1965; Seger and Brockmann, 1987; Frank and Slatkin, 1990; Leimar, 2005). However, this conclusion is based on the (implicit) assumption of a close correspondence between the phenotypes of parents and their offspring. We find a moderate and variable degree of mother–offspring resemblance of colour pattern in *Tetrix undulata* and *T. subulata* and propose that this variability in colour pattern among siblings may promote the maintenance of the polymorphism in temporally variable environments where relative fitness of alternative morphs changes. Suppose for the sake of argument that, under the conditions prevailing during a certain year, females belonging to the black colour morph enjoy a selective advantage. As a consequence of the low parent–offspring resemblance and pronounced heterogeneity among siblings, however, less than half of the offspring born to black females will belong to the black colour morph. The remaining progeny will comprise 'selective free-riders' that contribute to the preservation in the next generation also of alternative colour morphs that were not favoured by selection during that particular year. This corresponds to the weakening effect of low heritability on the evolutionary response to selection seen on quantitative characters.

In conclusion, our findings indicate that females may obtain fitness benefits mediated via increased diversification and viability of their hatched offspring. We also propose that offspring diversification provides an opportunity for selective free-riders that may broaden the conditions under which polymorphisms are possible.

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