

Dynamics of colour polymorphism in a changing environment: fire melanism and then what?

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Abstract Studies of whether disturbance events are associated with the changing genetic compositions of natural populations may provide insights into the importance of local selection events in maintaining diversity, and might inform plans for the conservation and protection of that diversity. We examined the dynamics of a colour pattern polymorphism in a natural population of pygmy grasshoppers *Tetrix subulata* (Orthoptera: Tetrigidae) inhabiting a previously burnt clear-cut area. Data on morph frequencies for wild-caught and captive-reared individuals indicated that the initial dominance of black phenotypes following the fire event was followed by an increased diversity of the polymorphism. This was manifested as the appearance of a novel morph, a decreased incidence of the black morph, and a more even distribution of individuals across alternative morphs following the recurrence of vegetation. We also found that the colour patterns of captive-reared individuals resembled those of their parents and that the degree of within-clutch diversity increased between generations. Our comparisons of morph frequencies across generations and between environments within generations point to a genetic determination of colour pattern, and indicate that the polymorphism is influenced more strongly by selection than by plasticity or migration.

Keywords Environmental disturbance · Evolution · Genetic variation · Insect · Selection

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Introduction

Environmental perturbations, such as forest fires, constitute natural experiments that may provide excellent opportunities to investigate the ecological and genetic consequences of environmental change. It is important to understand the effects of these stochastic disturbance events on genetic polymorphisms in natural populations for several reasons. First, there is currently no consensus in terms of theoretical predictions; some models predict that environmental fluctuations will erode the genetic variability of populations, whereas other models instead propose that temporal variation in selection will promote the maintenance of polymorphisms. These conflicting outcomes partly reflect differences in the assumptions (e.g. haploid versus diploid, overlapping versus non-overlapping generations) of the genetic models used (Frank and Slatkin 1990; Hedrick 1986; Leimar 2005; Levins 1968; Roff 1997; Seger and Brockmann 1987). For instance, although polymorphism is unlikely to be maintained by temporal variation in haploid systems, temporal variation can easily maintain polymorphism under diploidy (Seger and Brockmann 1987). Second, it is crucial to understand how environmental disturbances influence the genetic composition of natural populations for a variety of organisms if we are to develop successful plans for conserving and protecting biodiversity (Lande 1988; Soulé and Wilcox 1980). Moreover, while the study of evolution in heterogeneous environments is rich in theoretical models, there is a scarcity of data that can be used to empirically evaluate the theory (Roff 1997). Studies of how natural populations respond to environmental disturbances are thus of interest not only from theoretical and evolutionary viewpoints, but also from the more applied perspective of conservation.

Fire melanism in insects constitutes a useful model system for exploring effects of environmental change on

genetic variation in natural populations. Fire melanism refers to the rapid increase in the relative frequencies of black (melanistic) and dark-coloured phenotypes in populations living in recently fire-ravaged areas (Hocking 1964; Majerus 1998; Roff 1997; Rowell 1971). Three mechanisms have been proposed to account for the increased proportion of black phenotypes: natural selection (black phenotypes enjoy superior survival because they are better camouflaged against a black visual background); phenotypic plasticity (black coloration is induced by post-fire environmental cues, such as via homochrome response to the black background), and differential immigration and habitat selection (black phenotypes selectively move into fire-ravaged areas) (Hocking 1964; Rowell 1971). Several studies have documented increases in the relative frequencies of black phenotypes following fire events, and attempts have been made to identify the underlying mechanism(s) (Rowell 1971), but little is known about the more long-term consequences of fire events for the dynamics of polymorphisms.

Here, we use data from a natural population of colour polymorphic pygmy grasshoppers *Tetrix subulata* (Orthoptera: Tetrigidae) in a recently burnt clear-cut area to examine the dynamics of the polymorphism several years after the fire event. Pygmy grasshoppers are small (up to 15 mm in body length, 0.07 g), diurnal, ground-dwelling insects mostly inhabiting tropical rainforests but also temperate and even arctic regions of Europe, Asia and America. They feed on plant material such as algae, mosses and detritus. In disturbed habitats such as recently burnt clear-cut areas, populations may increase in numbers and thrive for a few years before declining rapidly again to low background levels following the recurrence of vegetation (Forsman and Appelqvist 1999; Holst 1986).

Pygmy grasshoppers exhibit a multitude of colour morphs within populations. Individuals may vary from black, through yellowish-brown, to light grey, with some being monochrome and others having distinct patterns (Fisher 1930, 1939; Forsman 1999; Good 1941; Nabours 1929). In some tetrigids, colour morphs represent alternative ecological strategies that also differ in traits such as body size, inter-clutch interval, body temperature preferences and thermoregulatory behaviour, anti-predator behaviour and microhabitat selection (Ahnesjö and Forsman 2003, 2006; Forsman 1999, 2000; Forsman and Appelqvist 1999; Forsman et al. 2002, and references therein). Some of these associations are based on phenotypic correlations only, but the 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 polymorphism in pygmy grasshoppers thus provides a proxy for the genetic variability of the population that is directly visible to the human eye.

The population investigated in this study inhabits a clear-cut field in south-central Sweden that was intentionally burnt for conservation purposes. We present data on colour morph frequencies in the population: (1) among free-ranging individuals collected as adults in two consecutive years; and (2) among their offspring that were raised in captivity and that did not encounter any burned materials during captive rearing. Using these data we examine changes in morph frequencies both across generations and between environments. Specifically, we address the following questions:

1. Do colour morph frequencies change between generations among free-ranging individuals? Such a shift would be consistent with each of the three hypotheses (evolutionary response to environmental change, phenotypic plasticity and differential immigration and habitat preferences).
2. Do colour morph frequencies differ between generations among captive-reared offspring born to wild-caught individuals? Such a difference would be consistent with the hypotheses of evolutionary change and phenotypic plasticity.
3. Do colour morph frequencies differ between individuals that developed under natural conditions and individuals from the same generation that were raised in captivity? Such a difference would be consistent with both the hypothesis of environmentally induced phenotypic plasticity of colour morphs and with the hypothesis of differential selection of colour morphs among free-ranging individuals.
4. Do colour morph frequencies differ between wild-caught individuals and their captive-reared offspring? The hypothesis of phenotypic plasticity predicts large differences between the two samples, whereas an overall correspondence/resemblance would be more consistent with the notion that colour morphs are genetically determined.

Materials and methods

Study population

Adults and late nymphal instar pygmy grasshoppers overwinter and emerge in spring (April–May in our study area) when the mating season begins. Females produce multiple egg pods (each pod containing <35 eggs) but probably do not survive for more than one reproductive season (Ahnesjö and Forsman 2003; Forsman 2001; Forsman and Appelqvist 1999). Nymphs develop through five (males) or six (females) instars before eclosing into the adult form.

The population of *Tetrix subulata* under investigation inhabited a large (~50 ha) clear-cut field located in the

vicinity of the village of Kosta, south-central Sweden (N56°51'25", E15°35'10"). The study site was intentionally burnt for management and conservation purposes in June 2003. The populated part of the area measured approximately 200 m × 200 m and consisted of a small wetland and a surrounding 20-m-wide corridor characterised by burnt organic matter, bare rocks and boulders, fallen logs and branches and live vegetation dominated by mosses (e.g. *Ceratodon purpureus*, *Pholia nutans*, *Sphagnum* ssp., *Polytrichum* ssp.), grass and some tussock sedges (*Carex* spp.). Grasshoppers were caught by hand by three or four people who simultaneously walked slowly through the area on two occasions, 25 May 2005 and 9 May 2006. All sighted adult individuals were caught and brought to the laboratory where they were sexed, classified by colour morph and housed for egg-laying, as described below.

Colour morphs

There are remarkable variations in the colours and patterns of the pronotum, legs and other parts of the body. Ground colours are black, light grey, dark brown, pale brown, reddish-brown, yellowish-brown or olive green. Some morphs are monochrome but others have pattern elements consisting of several widths and colours of longitudinal stripes along the median pronotum or on the femora of the jumping legs, or specks or spots about the middle or periphery of the pronotum or on the jumping legs. Morphs also vary with regard to the texture of the integument on the pronotum and the femur of the jumping legs, the surface being either smooth, or granular and rough, or consisting of longitudinal ridges and grooves (veining).

We classified individuals as belonging to one of nine colour morphs (depicted in Fig. 1): black (uniform black or black but with barely discernible markings on the dorsal surface of the pronotum); mottled black (black but with greyish or brownish markings dorsally, ventrally and on the femur of the jumping hind-legs); brown (mostly uniform brown dorsally and black laterally and ventrally); striped (black with a narrow mid-longitudinal yellowish stripe along the upper surface of the pronotum); striated (uniform pale clay-brown dorsally but with distinct texture consisting

of longitudinal ridges and valleys along the upper surface of the pronotum and along the femur of the hind-legs); grey (light grey to almost white dorsally, sometimes with small triangular black markings, and black laterally and ventrally); light/pale brown (uniform pale brown dorsally and on the femur of the jumping legs, black laterally and ventrally); mottled (cream to pale yellowish brown with darker brown spots dorsally, laterally and on the femur of the jumping legs, black ventrally); and mottled brown (brown but with black or greyish markings dorsally, laterally and on the femur of the jumping hind-legs). Some variation also exists within colour morphs. For instance, individuals may or may not have distinct white markings on the femur of the jumping legs (see the grey morph in Fig. 1). Furthermore, individuals may appear greenish or olive, and gradually appear to turn darker and change colour over time because they become covered with growth of algae (Helfer 1987).

Captive rearing

In addition to gathering information on relative morph frequencies among adult wild-caught individuals that had developed under natural conditions, we raised offspring of the wild-caught females in captivity. This provided us with information on relative morph frequencies among individuals that had developed under conditions of relaxed selection and allowed us to quantify and compare among-sibling diversity of colour patterns between generations.

We brought adult *Tetrix subulata* females collected on 25 May 2005 to the laboratory where we housed them individually (we assumed they had mated prior to capture) for egg-laying in 10-l plastic buckets containing a peat–soil mixture and maintained out-of-doors during summer. We watered the buckets at regular intervals to prevent the substrate from drying and to promote the growth of algae and mosses. On 5 September, we examined the buckets for offspring, which were counted (mean number of siblings per litter = 9.7, SD = 7.12, range 1–29 individuals, $n = 46$ families) and classified by colour morph.

On 9 May 2006 we again collected females from the same population and brought them to the laboratory for egg-laying. We housed these females in individual plastic



Fig. 1 The nine alternative colour morph categories of *Tetrix subulata* used in the study (from left to right): black, mottled black, brown, striped, striated, grey, light brown, mottled, and mottled brown

containers measuring 150 × 85 × 210 mm maintained in the laboratory at 28 ± 1 °C and 75% relative humidity in a light 12 h:dark 12 h cycle. Four fluorescent strip lights (Philips Master TL 28 W/830 HE, Amsterdam, The Netherlands) 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., Morristown, NJ, USA) to maintain air humidity at a stable level. We supplied each cage with 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). Complementary food was a slice of fresh potato. We examined the cages for egg pods every week. 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. After hatching we transferred and reared the nymphs by family in separate, white 10-l plastic buckets, as in 2005. On 17 July we examined buckets, and counted (mean number of siblings per family = 7.1, SD = 3.24, range 2–14 individuals, $n = 46$ families) and classified the grasshoppers by developmental stage (nymph or adult) and colour morph.

Data set and statistical analyses

Our data set consists of information on colour morph for 1,093 individuals representing two different generations of both free-ranging and captive-reared individuals, distributed as follows:

1. wild-caught adults ($n = 97$, 73 females, 24 males) collected in the field on 25 May 2005;
2. captive-reared offspring ($n = 450$) from 46 families (born to the above wild-caught females) that were inspected for colour morphs on 5 September 2005;
3. wild-caught adults ($n = 221$, 157 females, 64 males) collected in the field on 9 May 2006; and
4. captive-reared offspring ($n = 325$) from 46 families (born to the above wild-caught females) that were inspected for colour morphs on 17 July 2006.

We first tested for independence among colour morph, year and category of animals (wild-caught versus captive-reared) using log-linear analysis (Bishop et al. 1974; Sokal and Rohlf 1981) implemented using the CATMOD subroutine in SAS (SAS 2004). Tests of independence were based on comparisons of fit between a model containing and a model lacking the interaction of interest using log-likelihood ratio statistics. Because our results revealed complete interdependence among the three variables (see below) we continued to test for differences in morph frequencies between years and categories of animals by performing pair-wise tests of independence within each state using contingency table analyses (χ^2 tests).

To enable quantitative comparisons of differences in colour morph frequencies between samples we computed an index of dissimilarity. This index was defined as the sum of the absolute values of the pair-wise differences (computed for each morph) in relative morph frequencies between two samples. The index of dissimilarity thus describes the magnitude of the overall difference and yields a value of zero for two samples with identical morph frequency distributions. A major advantage of this index is that it is independent of sample size (unlike, e.g. the χ^2 statistic).

Results

Overall our analyses uncovered differences in relative frequencies of the nine alternative colour morphs across generations and between environments within generations. We also found a correspondence in morph frequencies between wild-caught adults and their captive reared offspring. The distribution of individuals across the nine colour morphs depended in a complex manner on year and category of individuals (wild-caught or captive-reared), as evidenced by results of log-linear analyses (Fig. 2; Table 1). An attempt to simplify the saturated model (which included the three-way interaction between colour morph, year and category of animals, all combinations of pair-wise interactions and all main effects, i.e. assuming complete interdependence) by excluding the three-way interaction resulted in a significant discordance between the reduced model and data (as evidenced by the significant likelihood ratio test of the model: $\chi^2 = 23.21$, $df = 7$, $P < 0.0016$). In the remainder of the “Results” section we therefore address the questions under investigation and test for independence of colour morph frequencies on year and category of animals using pair-wise contingency table analyses.

Comparing morph frequencies between generations: wild-caught individuals

A comparison between wild-caught adults collected in 2005 versus 2006 revealed that the distribution of individuals among the nine alternative colour morphs changed between generations ($\chi^2 = 28.85$, $df = 8$, $P < 0.0003$, dissimilarity index = 0.53). The change consisted of a decrease in the relative frequencies of black (from 47 to 32%, $\chi^2_1 = 6.78$, $P = 0.0092$), striped and grey individuals and increased frequencies of mottled brown (from 0 to 14%) and mottled black individuals from 2005 to 2006 (Fig. 2). As a consequence of these changes, the total number of colour morphs increased and the distribution of individuals among the alternative morphs became more even.

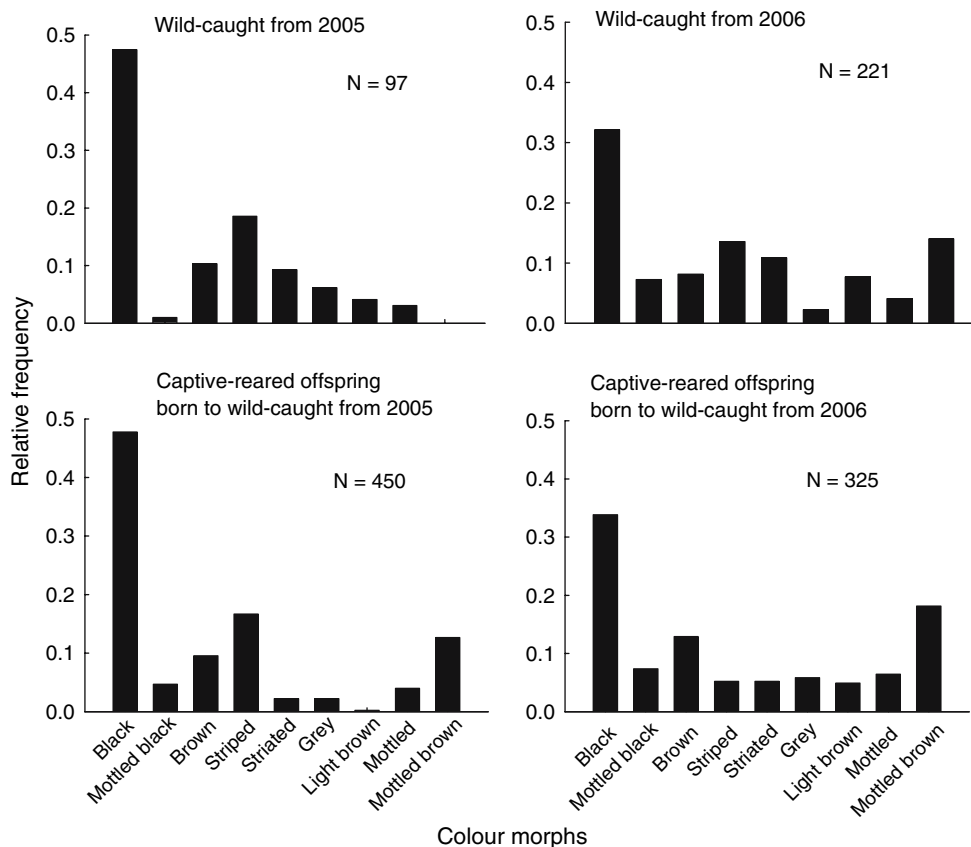


Fig. 2 Relative frequency distributions of nine alternative colour morphs in wild-caught and captive-reared samples of *Tetrix subulata* from 2005 and 2006. See text for details

Table 1 Results from tests of independence of *Tetrix subulata* colour morph frequency on year (2005 versus 2006) and category of animals (wild-caught versus captive-reared)

Source	df	Chi-square (χ^2)	P
Morph	8	605.25	<0.0001
Year	1	32.77	<0.0001
Category	1	80.48	<0.0001
Morph by year	8	58.87	<0.0001
Morph by category	8	27.86	0.0005
Year by category	1	45.40	<0.0001
Likelihood ratio	7	23.21	0.0016

Results are based on log-linear analysis of data for 1,093 individuals as implemented using the procedure CATMOD in SAS. The results shown are for a reduced model without three-way interaction. The significant likelihood ratio statistic of the model shows that the three-way interaction is statistically significant

Comparing morph frequencies between generations: captive-reared individuals

The comparison between captive-reared individuals from 2005 versus 2006 revealed that the distribution of individuals among the nine alternative colour morphs

changed between generations ($\chi^2 = 70.48$, $df = 8$, $P < 0.0001$, dissimilarity index = 0.51). The change consisted of a decrease in the relative frequencies of black (from 48 to 34%, $\chi^2_1 = 15.04$, $P < 0.0001$) and striped individuals and increased frequencies of light brown and mottled brown individuals from 2005 to 2006 (Fig. 2). The total number of colour morphs increased and the distribution of individuals among the alternative morphs became more even in 2006 compared to 2005, as in wild-caught individuals.

Comparing morph frequencies within the same generation: free-ranging versus captive-reared individuals

A comparison between adults collected in the field in 2006 versus individuals that belonged to the same generation but had been raised in captivity revealed that the distribution of individuals among the nine alternative colour morphs was dependent upon rearing conditions ($\chi^2 = 64.40$, $df = 8$, $P < 0.0001$, dissimilarity index = 0.40). The difference consisted of a lower relative frequency of the black morph and lower frequencies of the striated and light brown morphs among offspring born in 2005 that had grown up in the wild compared to the captive-reared individuals (Fig. 2).

Comparing morph frequencies among wild-caught individuals and their captive-reared offspring

The comparisons of morph frequencies in wild-caught adults versus their offspring that had been raised in captivity revealed minor albeit statistically significant differences in the distribution of individuals among the nine alternative colour morphs between the parental and offspring generations both in 2005 ($\chi^2 = 44.31$, $df = 8$, $P < 0.0001$, dissimilarity index = 0.35) and in 2006 ($\chi^2 = 27.28$, $df = 8$, $P < 0.0006$, dissimilarity index = 0.33, Fig. 2). In 2005 the change consisted of a decline in the frequencies of striated and light brown individuals and an increased frequency of mottled brown individuals from parents to offspring. In 2006 the change consisted of a decline in the frequencies of striated and striped phenotypes and increased frequencies of grey and mottled brown phenotypes from parents to offspring. Compared to the situation for wild-caught individuals, the magnitude of the difference in morph frequencies between captive-reared individuals and their parents was smaller (as evidenced by the lower index of dissimilarity). The overall impression from a visual inspection of histograms is that the correspondence between the parental and offspring generation is relatively high, particularly with regard to the black colour morph (Fig. 2).

Within-family diversity and parent-offspring resemblance

Analysis of data based on offspring born to females mated in the field prior to capture uncovers considerable diversity among siblings from within the same clutch with regard to colour morph both in 2005 (median = 2.5, mean = 2.6, SD = 1.3, range 1–5 colour morphs, $N = 46$ clutches, Fig. 2) and in 2006 (median = 3.0, mean = 2.8, SD = 1.1, range 1–5 colour morphs, $N = 46$ clutches, Fig. 3). The average number of colour morphs per family did not differ between years (Wilcoxon two-sample test, normal approximation: $Z = 1.06$, $P = 0.29$), but this comparison is somewhat flawed because average family size was larger in 2005 than in 2006 (9.5 versus 7.1 surviving offspring at the time of the late summer inventory, see “Materials and methods” for details, $t_{90} = 2.13$, $P < 0.05$). Within-family diversity, measured as number of colour morphs, was significantly lower in 2005 than in 2006 when the difference in family size between years was statistically controlled for by including family size as a covariate (ANCOVA: least squares means 2005 = 2.43 morphs, 2006 = 2.93 morphs, effect of year: $F_{(1,89)} = 6.45$, $P < 0.05$).

Parent-offspring resemblance (measured as the percentage of offspring within a clutch that belonged to the same colour morph as their mother) in colour pattern was 32% (SE = 4.6%, range 0–100%, $N = 46$ families) in 2005 and 41% (SE = 4.9%, range 0–100%, $N = 46$ families) in 2006.

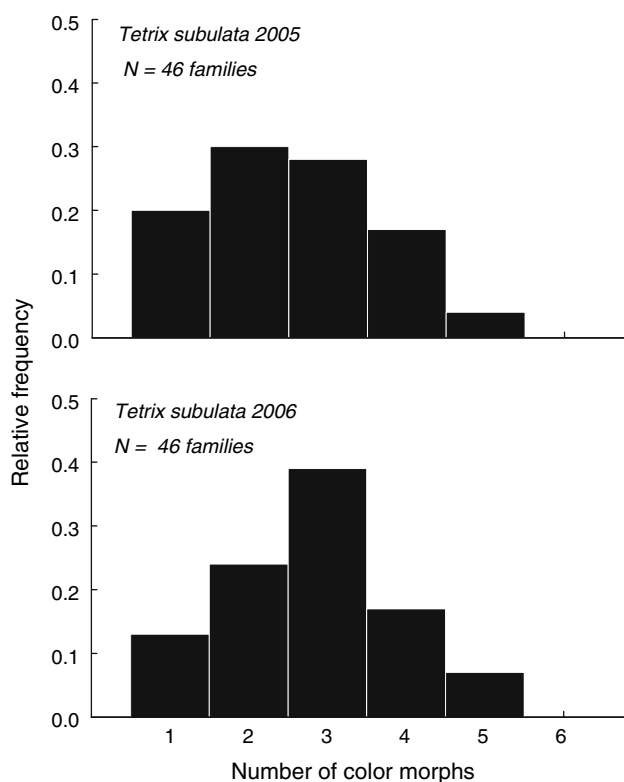


Fig. 3 Relative frequency distributions of clutches as a function of number of colour morphs available among siblings within the same clutch. Data for *Tetrix subulata* families raised in captivity and born to females collected as adults (mated in the field prior to capture) from a natural population in 2005 and 2006

Because there was no difference between years ($t = 1.07$, $df = 90$, $P = 0.29$) we pooled data and arrived at a common estimate of parent-offspring resemblance of 37% (SE = 3.4%, range 0–100%, $N = 92$ families). Females of the black colour morph produced a larger percentage of black offspring compared with non-black females, and the percentage black offspring was higher on average in 2005 than in 2006 (two-factor ANOVA on arcsine-square-root-transformed proportions, effect of maternal colour morph (black versus non-black): $F_{(1,89)} = 4.71$, $P < 0.05$; effect of year: $F_{(1,89)} = 5.93$, $P < 0.05$, Fig. 4).

Discussion

Overall our analyses of colour polymorphism in wild-caught and captive-reared individuals from a population of pygmy grasshoppers inhabiting a previously fire-ravaged area indicated that the initial increase in relative frequency of black phenotypes was followed by an increased diversity of the polymorphism over time. This was manifested as the appearance of a novel morph with a concomitant increase in the total number of colour morphs in the population, a

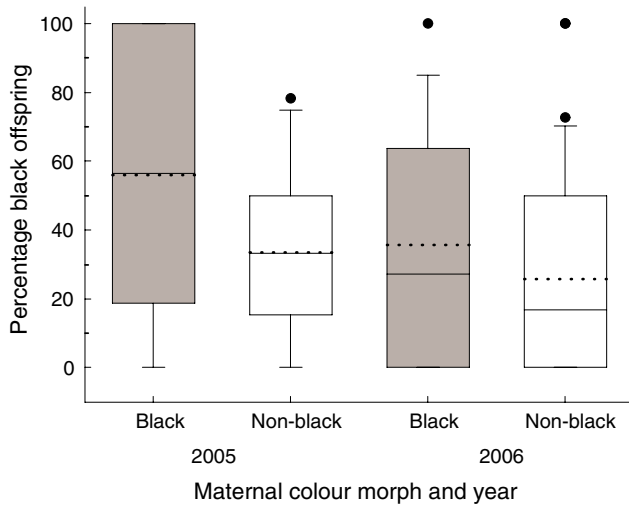


Fig. 4 Comparison of percentage of siblings that belonged to the black colour morph among captive-reared offspring produced by either black or non-black mothers in 2005 and 2006. Figure is based on family means. The boundaries of the *box* indicate the 75th and 25th percentiles, the *solid* and *dotted* lines within the *box* mark the medians and means, and *whiskers* above and below indicate the 90th and 10th percentiles

decreased incidence of the black morph, a more even distribution of individuals across the nine alternative morphs, and even an increased degree of within-clutch diversity (Figs. 2, 3). We found that colour morph frequencies changed between generations in free-ranging as well as in captive-reared individuals, differed between wild-caught individuals and their captive-reared offspring, and differed between individuals that had developed under natural conditions and individuals from the same generation that were raised in captivity. Collectively, these findings enable us to evaluate the relative contributions of phenotypic plasticity, selection and gene flow to the observed dynamics.

The relative contributions of phenotypic plasticity and genetics to colour morph determination

If phenotypic plasticity played an important role in the determination of colour pattern of pygmy grasshoppers one would expect considerable differences between individuals that were raised in plastic buckets in captivity and individuals from the same cohort that grew up under natural conditions. Although morph frequencies differed somewhat between the two samples, the overall correspondence between individuals raised in captivity and the wild is difficult to reconcile with the plasticity hypothesis. For instance, the relative frequency of the black morph was lower among offspring born in 2005 that grew up in the wild where they were exposed to burnt black substrates (i.e. wild-caught from 2006) than for captive-reared individuals of the same generation that had never experienced burnt

substrates (32 versus 48%; Fig. 2). We also found a high correspondence of morph frequencies between wild-caught parental and captive-reared offspring generations, particularly with regard to relative frequency of the black morph (2005: 47 versus 48%, 2006: 32 versus 34%, Fig. 2). Both of these findings are at odds with the hypothesis that fire melanism in pygmy grasshoppers reflects phenotypic plasticity in response to background colour. Earlier investigations have also failed to detect environmentally induced plasticity of coloration in congeneric species *T. undulata* (Ahnesjö and Forsman 2003; Forsman et al. 2002). The conclusion that plasticity is relatively unimportant conforms with the results of Nabours (1929), who bred two other species of tetrigids (*Paratettix texanus* and *Apotettix eurycephalus*) for more than 20 years and 60 generations and found no plasticity of coloration in response to environmental effects: “neither excessive humidity, temperature, aridity, acidity, salinity, sunlight through glass or direct, darkness, colour of soil, food, excreta, starvation, fungus disease, parasitism, nor any other observable feature of the environment has ever changed colour pattern to any appreciable extent, or in any way influenced those of the progeny” (Nabours 1929).

The overall similarity in the present study of the relative frequency distributions of colour morphs in wild-caught animals and their captive-reared offspring adds further support to the conclusion that colour patterns in pygmy grasshoppers are genetically determined (Ahnesjö and Forsman 2003; Fisher 1930, 1939; Forsman et al. 2002; Nabours 1929). The higher percentage of black offspring produced by black compared with non-black mothers (Fig. 4) also suggests a genetic component to colour pattern determination. Our estimate of parent offspring resemblance (an index of heritability) in colour pattern was 37%. This is almost identical to the estimate for *T. undulata* (36%, SE = 3.4%, range 0–100%, $N = 75$ families; A. Forsman et al., unpublished data). This moderate parent-offspring resemblance and considerable diversity with regard to colour morph among siblings within the same clutch (Fig. 3) may stem from the fact that female pygmy grasshoppers are polyandrous (Caesar et al. 2007) such that siblings within the same clutch are sometimes sired by different males (Nabours 1929; A. Forsman et al., unpublished data). The genetic contribution to colour pattern determination raises the possibility that the documented shifts in colour morph composition between generations reflect microevolutionary changes caused by selection, gene flow or random events.

Selection

We found that the black morph was less frequent in the sample of free-ranging individuals than in the individuals

from the same cohort that had been raised in captivity (32 versus 48%; Fig. 2). This suggests that the decrease in the frequency of the black morph in the population from 2005 to 2006 may have reflected selection against black phenotypes. That the decline of the black morph coincided with the recurrence of vegetation and a concomitant reduction in the proportion of burnt, black substrate further suggests that differential predation may have contributed to the observed changes in morph frequencies. Vulnerability to predators and parasitoids is determined by complex interactive effects of animal pigmentation pattern, background coloration, ambient light conditions and the visual perceptive abilities of the beholder (Endler 1978, Endler 1990) in combination with antipredator behaviours, such as selection of habitats that reduce the risk of detection (Cott 1940; Isley 1938). Earlier work on pygmy grasshoppers suggests that different morphs vary in susceptibility to visually oriented predators and underscores the importance of the combined effects on predation risk of coloration, behaviour and changing visual backgrounds (Ahnesjö and Forsman 2006; Civantos et al. 2004; Forsman and Appelqvist 1998, 1999). The higher frequency of the striped morph in the field (versus captivity) may reflect low susceptibility to predation due to the protective value of disruptive coloration (Ahnesjö and Forsman 2006; Cott 1940).

Investigations into developmental instability (manifested as unusually high levels of fluctuating asymmetry) of pygmy grasshoppers suggest that the black morph may be of low inherent quality (Civantos et al. 2005). Perhaps the increased frequency of black phenotypes in recently fire ravaged areas is best explained by strong selection against all colour morphs other than the black one. In keeping with this interpretation, the black morph may flourish in recently burnt areas (despite being of inferior inherent quality) due to a temporary selective advantage associated with superior crypsis, but then decline in frequency again as a result of changing microhabitat composition and regained relative crypsis (and fitness) of alternative colour morphs (see also Ford 1945 and Majerus 1998 for a similar argument for industrial melanism in butterflies).

Gene flow and differential habitat preferences

The consequences of occasional disturbance events such as forest fires for the genetic composition of populations are not limited to effects caused by temporal variation in selection. The increased spatial heterogeneity (i.e. comparing burnt versus adjacent unburnt areas) caused by fire events may also play an important role. Indeed, spatial variation in environmental composition and selective regimes in combination with gene flow are probably the main determinants of the level of genetic polymorphisms (Hedrick 1986; Levene 1953). Unfortunately we do not have the informa-

tion on dispersal capacity of pygmy grasshoppers required to evaluate the role of gene flow in the dynamics of the polymorphism in this system. Available mark–recapture data and behavioural observations of free-ranging individuals suggest that pygmy grasshoppers are sedentary animals that perform short-distance movements of on the order of a few metres per day (Ahnesjö and Forsman 2006; Forsman and Appelqvist 1999). Furthermore, colour morphs differ in habitat selection at the small spatial scale, but the overlap is considerable (Ahnesjö and Forsman 2006). We therefore consider it unlikely that differential immigration and habitat preferences are responsible for the observed changes in morph frequencies, but this conclusion is tentative only.

Interactive effects of population dynamics and population genetics

Pygmy grasshopper populations in recently burnt areas initially explode in numbers and thrive for a few years before declining rapidly again to low levels following the recurrence of vegetation and recolonization of other invertebrates. How could these fluctuations influence the evolutionary dynamics of the polymorphism? The conventional view is that small population size results in reduced genetic variability, as exemplified by losses of allozyme alleles (Dobzhansky 1970; Ford 1964; Hedrick 1986). On the other hand, theoretical models and experimental evidence suggest that bottlenecks and founder events (inbreeding) may also cause shifts in the organization of the gene pool (e.g. conversion to additive of a portion of the epistatic variance, disruption of covariance matrices) and lead to increased genetic variance of quantitative traits (Barton and Turelli 2004; Björklund 2004; Briggs and Goldman 2006; Carson 1990; Whitlock et al. 2002). Selection and evolutionary changes in genotype frequencies also may have reciprocal effects on population dynamics (Ford 1945; Saccheri and Hanski 2006; Yoshida et al. 2003). At the extreme level, genetic variation may influence the probability of extinction either by decreasing effects of detrimental variants or by decreasing the potential for future adaptive responses (Hedrick et al. 2006; Kolbe et al. 2004). Pygmy grasshoppers may offer an additional candidate example. The association of colour patterns with other morphological, behavioural and life-history traits means that the different morphs represent alternative strategies or eco-morphs (see above for references). This makes the occurrence of multiple morphs among siblings within a single clutch particularly interesting. We have evidence indicating that a female may increase the offspring diversity by mating with more than one male (A. Forsman et al., unpublished data). This raises the possibility that a single successful “founder” gravid female mated to several males may carry over much of the original genetic variability (plus perhaps the positive

effect of founder events on additive genetic variance) and thereby provide an important source of genetic variation from which natural selection may build novel character states in local populations.

In conclusion, our analyses of wild-caught and captive-reared pygmy grasshoppers from a population inhabiting a previously burnt area revealed that colour morph frequencies changed between generations, and that the initial dominance of black phenotypes following the fire-event was followed by an increased diversity of the polymorphism following the recurrence of vegetation. Collectively, our comparisons across generations and environments suggest a genetic determination of colour pattern and indicate that the polymorphism is influenced more strongly by selection than by plasticity or migration. These findings also are indicative of reciprocating effects of the genetic composition and the dynamics of natural populations that may have important implications for the protection of biodiversity (Forsman et al. 2007). More long-term studies are nevertheless needed to shed additional light on the influence of environmental disturbances on the variability and organization of the gene pool in natural populations. We propose that fire melanism in insects may provide one fruitful avenue for future research in this field.

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