

Dietary differences among colour morphs of pygmy grasshoppers revealed by behavioural experiments and stable isotopes

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ABSTRACT

Question: Do alternative colour morphs differ in food preferences and realized dietary niches?

Hypothesis: Colour morphs represent alternative phenotypes that exploit different dietary niches due to combined effects of differences in food preferences and physiological demands, availability and spatial distribution of alternative food types, and morph-specific microhabitat utilization.

Organisms: Adult female *Tetrix subulata* pygmy grasshoppers belonging to one of three colour morphs: dark, pale or striped. Colour pattern in these grasshoppers is genetically influenced and barely, if at all, affected by developmental plasticity in response to environmental cues. *Tetrix subulata* inhabits damp places, primarily on the soil surface, and feeds on algae, short grass, moss, and humus. We collected and studied individuals of seven populations in southeast Sweden: three from pastures, two from alkaline fens, and two from burnt clear cuts.

Methods: First, we performed two multiple-choice feeding experiments: (1) between individuals from five different source populations, and (2) between individuals of different colour morphs from the same population. Second, we measured natural abundances of carbon and nitrogen stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in free-ranging individuals from two populations to test for differences in long-term diets among populations and colour morphs.

Results: In the multiple-choice feeding experiments, utilization of food types differed both among populations and among colour morphs within a population. The comparisons of stable isotope ratios indicated long-term differences in diet both among populations and among colour morphs.

Conclusions: Food preferences and dietary niches differ among pygmy grasshopper populations and colour morphs.

Keywords: colour polymorphism, insect, niche, resource partitioning, *Tetrix subulata*.

INTRODUCTION

A natural population of a single species commonly contains individuals that exhibit a broad array of phenotypic variation related to sex, age, size, morphology, colour or behaviour (Roughgarden, 1972; Shine *et al.*, 2003; Svanback and Bolnick, 2007). This variation may be accompanied by different demands for environmental resources such as food or habitat, and it is increasingly recognized that different subsections of a population and even individuals may differ in their realized niches (Polis, 1984; Bolnick *et al.*, 2002, 2003). This phenomenon is likely to be accentuated in polymorphic species with dramatic distinct phenotypic forms in the population (Skulason and Smith, 1995). Differential niche use by discrete intra-specific morphs was termed 'resource polymorphism' by Smith and Skulason (1996), and defined as discrete differences in habitat use or feeding biology.

Resource polymorphism is well documented in vertebrates, including fish, amphibians, and birds (for reviews, see Wimberger, 1994; Smith and Skulason, 1996), and it has mostly been associated with differences in size and shape of mouth parts or other feeding structures. Differential resource use in colour polymorphic species has been primarily discussed in the context of crypsis and the effect of predator avoidance behaviours on microhabitat selection (see, for example, review by Bond, 2007). Studies of colour polymorphism in ectotherms further indicate that differently coloured individuals differentially use available microhabitats that provide protection against visual predators or offer thermally suitable conditions (e.g. Jones, 1982; Endler, 1983; Morey, 1990; Shine *et al.*, 1998; Ahnesjö and Forsman, 2006). Other studies have failed to detect differential habitat use by alternative colour morphs, including in sea snakes *Emydocephalus annulatus* (Shine *et al.*, 2003) and the marine herbivore isopod *Idotea balitica* (Merilaita and Jormalainen, 1997).

Studies of diet-related resource partitioning among alternative colour morphs are limited (Shine *et al.*, 1998; Wilson and Jamieson, 2005; Nosil *et al.*, 2006; Anthony *et al.*, 2008). Yet, there are several reasons why different colour morphs may be expected to utilize different subsets of available food resources (Forsman *et al.*, 2008). The diets of free-ranging animals are governed not only by individual preferences and active choice, but also by food availability and a series of competing demands and constraints (Pulliam, 1974; Futuyma and Peterson, 1985; Stephens and Krebs, 1986), and individuals that belong to different colour morphs or originate from different populations may vary in both realized and preferred diet (Arnold, 1977). For instance, different morphs may be active at different times of the day and therefore encounter different prey types (Anthony *et al.*, 2008). Individuals belonging to different colour morphs may encounter different food types if they vary in the way that they utilize available microhabitats (Futuyma and Peterson, 1985). For example, to avoid being detected by prey, different predator morphs may hunt in different environments that may contain different food items (Green, 2005). Conversely, selection for background matching imposed by predators may enforce prey morphs to different habitats with dissimilar food sources (Todd *et al.*, 2006). Similarly, if alternative colour morphs seek out microhabitats that provide thermally suitable conditions, they may encounter different food types as a by-product of body temperature regulation behaviour (Jones, 1982; Ahnesjö and Forsman, 2006). In addition, colour morphs may vary in their innate preferences, metabolism, and physiological demands for food. Individuals represent functionally integrated units, and in several colour polymorphic species colour pattern is developmentally, phenotypically, and genetically associated with physiology, morphology, behaviour, and life history (True, 2003; McKinnon and Pierotti, 2010).

To our knowledge, no attempt has been made previously to experimentally test for differences in food preference as well as long-term differences in diet among alternative

colour morphs of generalist or omnivorous species. Pygmy grasshoppers, *Tetrix subulata*, provide a suitable model system, since they exhibit distinct colour polymorphism. Ample available evidence indicates that colour pattern in pygmy grasshoppers is genetically influenced and barely, if at all, affected by developmental plasticity in response to environmental cues (Nabours, 1929; Karlsson *et al.*, 2009). Furthermore, colour morphs are genetically correlated with specific physiological, morphological, behavioural, and life-history characteristics, such that they represent ecomorphs (Forsman, 1997; Ahnesjö and Forsman, 2003, 2006), and they have been shown to differ in predator avoidance behaviour and microhabitat use (Forsman, 1997; Ahnesjö and Forsman, 2006 and references therein).

In this study, we combine information from behavioural feeding experiments and isotopic signatures of free-ranging pygmy grasshoppers to test the prediction (Forsman *et al.*, 2008) that individuals belonging to alternative colour morphs differ in food preferences and dietary niche divergence, and to elucidate how preferences and ecological constraints jointly contribute to dietary differences among colour morphs and populations. We use two approaches that provide slightly different and complementary information. First, we perform multiple-choice feeding experiments, a straightforward way to test for differences in food preferences. Second, we use naturally occurring stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), a standard way to investigate and compare diet because of high correlations between isotope signatures in the diet and those found in the consumer animal's tissue (e.g. Fry *et al.*, 1978). Isotopic signatures provide a means of analysing long-term (depending on the rate of tissue turnover) dietary differences in free-ranging animals in their natural environment, and reflect the food actually eaten but not necessarily preferred (Deniro and Epstein, 1978a, 1978b; Bearhop *et al.*, 2004; Hood-Nowotny and Knols, 2007).

METHODS AND MATERIALS

Study species

The pygmy grasshopper, *Tetrix subulata*, is a widely distributed insect that inhabits biomes ranging from tropical rainforests to arctic regions of Europe, Asia, and north to southern north America (Mexico), except the south-eastern United States (Rehn and Grant, 1955). *Tetrix subulata* is a small (<15 mm total body length, mean 0.07 g dry body mass), diurnal, ground-dwelling insect that most frequently inhabits damp places where it primarily lives on the soil's surface and feeds on algae, short grass, moss, and dead, partly decayed animal and plant matter in the litter (Holst, 1986). *Tetrix subulata* exhibits a large number of discrete colour morphs. Ground colours range from black, via various shades of brown, light grey to almost white. Some morphs are monochrome, whereas others have patterning consisting of a longitudinal stripe along the median pronotum or on the femora of the jumping legs, or of specks or spots, of variable colours and widths (Nabours, 1929; Karlsson *et al.*, 2008; Caesar *et al.*, 2010). The frequencies of alternative colour morphs in *Tetrix* vary among populations and over time within populations (Forsman *et al.*, 2011).

Multiple-choice feeding experiments

Two separate multiple-choice feeding preference experiments were performed. One addressed among-population differences and was carried out in 2009. The other addressed differences among colour morphs within a population and was carried out in 2010.

Experimental animals for comparisons among individuals from different populations

To test for differences in dietary preferences among individuals originating from different natural populations that inhabit different environments and have different evolutionary histories, adult female *T. subulata* were collected and tested in May 2009 from five populations in southeast Sweden [i.e. Hägern, forest pasture, 57°25.384N, 16°15.964E ($n = 11$); Flyvägen, burnt clear cut, 57°00.477N, 16°06.022E ($n = 14$); Ålem, pasture adjacent to a clear cut, 56°56.019N, 16°21.808E ($n = 13$); Jordtorp, alkaline fen, 56°40.613N, 16°33.335E ($n = 7$); and Bredsåtra, enclosed pasture, 56°50.962N, 16°47.367E ($n = 6$)]. The latter two populations are found on the island of Öland, while the first three are found on the mainland. We only used individuals that belonged to one of three different colour morph categories (i.e. dark, light, and striped). The dark category consisted of dark brown individuals, the light category consisted of grey/yellowish individuals, and the striped category consisted of individuals with a dark background accompanied by a yellow longitudinal stripe along the pronotum (for photographs of different morphs, see Karlsson *et al.*, 2008; Caesar *et al.*, 2010). Not all morphs were present at each location. However, the aim of this experiment was to test for dietary divergence among populations – we performed a separate experiment in 2010 to specifically investigate the divergence among colour morphs within a population (see below). Before the onset of the experiment (always the day following capture), the grasshoppers were housed in an aquarium and provided with water but no food. A total of 51 grasshoppers were used in this experiment.

Experimental animals for comparisons among colour morphs within a single population

To test for differences in dietary preferences among individuals belonging to different colour morphs, adult female *T. subulata* were collected and tested in mid-May 2010 from a single natural population in an alkaline fen habitat in Vanserbäck on the island of Öland (56°40.424N, 16°38.214E), southeast Sweden. Animals were brought to the laboratory and classified as belonging to one of three colour morph categories: dark ($n = 35$), light ($n = 32$) or striped ($n = 30$). The dark category consisted of dark brown individuals, the light category consisted of grey/yellowish individuals, and the striped category consisted of individuals with a dark background accompanied by a yellow longitudinal stripe along the pronotum (for photographs of different morphs, see Karlsson *et al.*, 2008; Caesar *et al.*, 2010). Before the onset of the experiment (always the day following capture), the grasshoppers were housed in an aquarium and provided with water but no food. A total of 97 adult females were used in the experiment.

General procedure of feeding experiments

The procedure for the feeding experiments was similar in both years, whereby each grasshopper was placed in a circular cage and presented with five different food items (Fig. 1). Each cage was constructed using a Petri dish (diameter 8.5 cm) as the bottom, and approximately 7-cm high 'Xerox transparent' as the wall. The bottom of each cage was padded with wet cotton to supply the grasshoppers with drinking water and to prevent the food items from drying out. The cage was sealed with the lid of the Petri dish that was pierced with a needle to allow a flow of air. In total, we used 30 cages. Previous investigations indicate that pygmy grasshoppers, including *T. subulata*, feed on mosses, algae growing on moist soils, and humus or detritus (Nabours, 1929; Ahnesjö and Forsman, 2006), and hardly ever on sprouting grasses or forbs (Hochkirch *et al.*, 2000). We therefore presented the

following five food items to the grasshoppers: the top of vernal sedge (*Carex caryophylla*, which included the pollen), the top of tufted sedge (*Carex elata*; 2009 experiment only), common hair moss (*Polytrichum commune*), carpet moss (*Mnium* sp.) and calliergonella moss (*Calliergonella cuspidata*), together with soil with microalgae. Similar-sized pieces of the five different food items were presented simultaneously (cafeteria style) in a circular manner along the inner wall but perpendicular to it with branches towards the centre of the cage. Food items were placed in random order in each cage, so that the different food items had similar probability to be near any other food item. The latter two mosses and the sedge were common at and collected from the grasshopper's natural habitat, and the soil (a bought mixture of peat and soil) and common hair moss (originating from nearby forest wetland) were collected from the rearing buckets used to breed grasshoppers in captivity. None of the food items was fully consumed during the observation period, and so grasshoppers were not forced to feed on less favoured items.

Behavioural observations and statistical analyses

In each of the 2009 and 2010 experiments, 3 days of observations were performed within a 10-day interlude. All grasshoppers were tested the day following capture. Experimental cages were placed in the laboratory approximately 20 cm below two lamps (Philips daylight, 60 W/230 V). The temperature was approximately 32°C inside the cages and in the room in which the experiment was conducted. In each session of the 2009 experiment, between 10 and 12 grasshoppers representing each of the five different source populations were randomly assigned to and placed individually inside separate experimental cages. In each session of the 2010 experiment, between 10 and 12 grasshoppers representing each of the three colour morph categories were randomly assigned to and placed individually inside separate experimental cages. At the onset of an experimental trial, one grasshopper was released in the middle of each cage and its choice was first recorded after 3 min of familiarization. For the comparisons among individuals that originated from different source populations (performed in 2009), we recorded the behaviour of each grasshopper every 3 min for 3 h, yielding a total of 60 observations per individual. A food item was recorded only when grasshoppers were observed eating or tasting it, otherwise it was indicated 'none'. Preliminary analyses of these data indicated that the behaviour of individuals did not change much over the course of the 3-h experimental trials. In the experiment performed in 2010 to test for differences among individuals that originated from the same population but belonged to different colour morphs, we therefore reduced the observation period and recorded the behaviour every 3 min for 1 h, yielding a total of 20 observations per grasshopper; however, we increased markedly (from 51 to 97) the number of individuals that were observed.

For each experiment, we used general linear mixed models, implemented using procedure MIXED (SAS, 2004) to test the null hypothesis that utilization by individuals of the five different food types was independent of source population (2009) or colour morph category (2010). Following the approach outlined by Elmer *et al.* (2010), utilization was used as a response variable with food type and population (2009) or food type and colour morph (2010) as fixed effects. With this approach, each individual contributes only one observation for each food type, although we made repeated observations of each individual during the experiment. The identification code of each individual was used as a random effect. A likelihood ratio test was used to assess the statistical significance of the interaction term between food type and population (2009) and food type and colour morph (2010). Data

on proportional utilization were $\log + 1$ transformed. We also conducted separate analyses for each food type, using one-way analyses of variance (ANOVA).

Since the 97 grasshoppers used for the multiple-choice feeding experiment in 2010 were deprived of food after capture, except for the 1 h of observation, these animals were sacrificed at the termination of the experiment and used to examine long-term differences in diet among colour morphs by analysing stable isotopic signatures, as described below (two individuals escaped, leaving 95 individuals).

Comparison of stable isotope ratios

We analysed and compared stable isotope signatures to test for long-term differences in diet among individuals belonging to different colour morphs using animals from two different source populations. We used the adult female *T. subulata* grasshoppers collected (in May 2010) from the Vanserumbäck population that were used in the single-population multiple-choice feeding experiment in 2010. In addition, we used adult *T. subulata* females collected in May 2010 from a second population on the mainland in southeast Sweden (Hovmantorp 56°47.167N, 015°10.039E). From this population we only have data for individuals that belonged to the black ($n = 17$) and striped ($n = 17$) morphs, since no light individuals could be found. The individuals from Vanserumbäck that were used in the behavioural experiment were starved for 24 h before the experiment and were sacrificed immediately after the 1 h observation period, so any food consumed during the experiment could not have influenced our isotopic results.

We collected samples of two distinct body parts (head and hind femur) from each grasshopper. Samples were oven-dried at 60°C for 48 h, ground to a fine powder, and weighed (approximately 1–3 mg) into tin capsules and sent to UC Davis Stable Isotope Facility (University of California, Davis, CA) for ^{13}C and ^{15}N analysis. Results are expressed as $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, defined as the ‰ deviation of the sample from international reference (i.e. Pee Dee belemnite carbonate for $\delta^{13}\text{C}$ or air for $\delta^{15}\text{N}$) by the equation:

$$\delta X = [(R_{\text{sample}}/R_{\text{reference}}) - 1] * 1000,$$

where $X = ^{15}\text{N}$ or ^{13}C , and R_{sample} and $R_{\text{reference}}$ are the corresponding isotopic ratios $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$ of the sample and the reference, respectively (Hood-Nowotny and Knols, 2007). The long-term precision for analysis (standard deviation of the internal standard) was $\pm 0.2\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 0.3\text{‰}$ for $\delta^{15}\text{N}$.

We used multivariate analysis of variance (MANOVA) models with type-III sums of squares to examine the partitioning of variance in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope signatures among body parts (hind femur vs. head), source populations, and colour morphs (Sokal and Rohlf, 1981; SAS, 2004). In this approach, measurements of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from each individual and body part were treated as a repeated measure.

RESULTS

Significant differences in preference of food types between individuals originating from different populations, and among colour morphs within populations, were revealed by our multiple-choice experiments. Those dietary differences were further supported by differences in natural abundance of $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ stable isotopes.

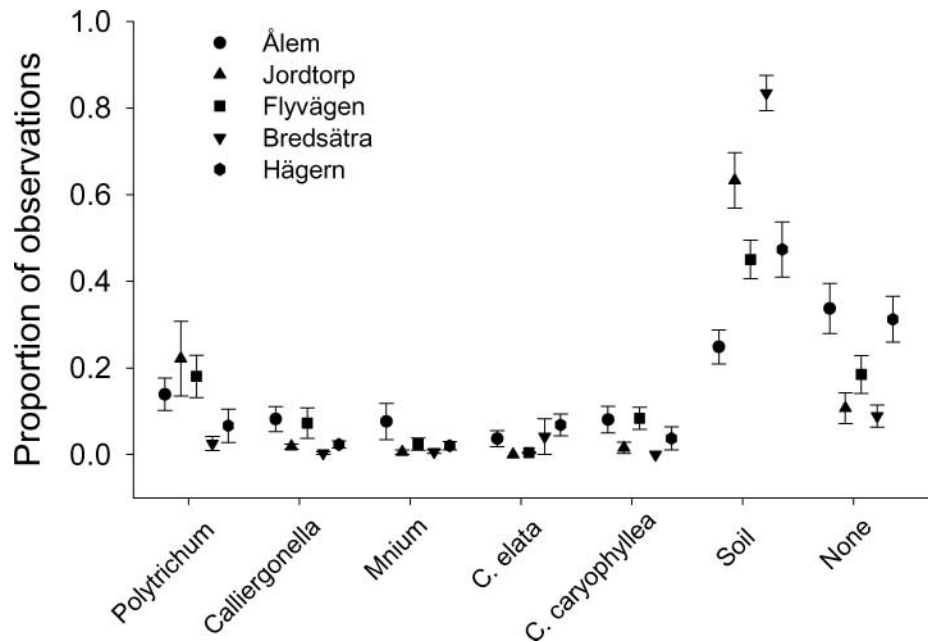


Fig. 1. Results from laboratory feeding experiment showing mean ($\pm$ S.E.) proportions of observations in which *Tetrix subulata* collected from five different source populations were observed eating each of five types of food plus soil with microalgae.

2009 feeding experiment: comparison between populations

Data on behaviour of individuals that originated from the five different source populations revealed that utilization of the five different food types was dependent on the grasshoppers' source population (as evidenced by a significant interaction between food type and population, $G = 107.1$, d.f. = 24, $P < 0.0001$) (Fig. 1). Soil containing microalgae was the most preferred food type by individuals from all populations, while all other food types were used to lesser extents (Fig. 1). Separate analyses for each food type further indicated that there were significant differences among the different populations in utilization of soil with microalgae, *Polytrichum* hair moss, and *Carex* sedge (all $P < 0.05$), but not in the utilization of *Calliergonella* or *Mnium* (both $P > 0.15$). Individuals from different source populations also differed in the extent to which they were observed on the cotton rather than on any of the different food types ($P < 0.05$) (Fig. 1).

2010 feeding experiment: comparison among colour morphs

Data on behaviour of dark, striped, and pale individuals that originated from the same source population revealed that utilization of the five different food types was dependent on grasshopper colour morph (effect of interaction between food type and colour morph, $G = 25.2$, d.f. = 10, $P < 0.005$) (Fig. 2), indicating that individuals belonging to different colour morphs showed differential preferences for the various food types. Visual inspection of the data revealed that soil containing microalgae was the most preferred food type by all

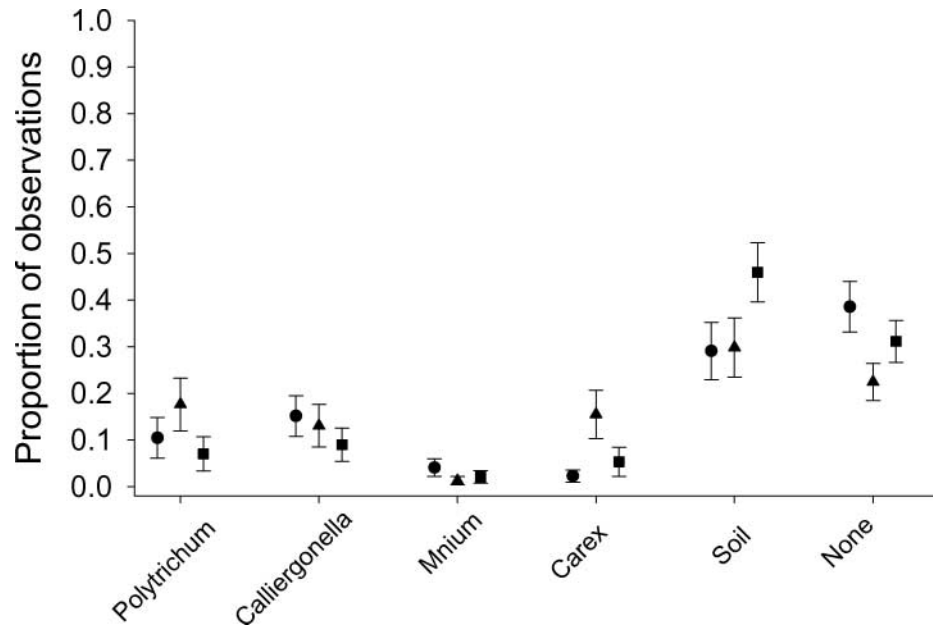


Fig. 2. Results from laboratory feeding experiment showing mean ($\pm$ S.E.) proportions of observations in which striped (●), pale (▲), and dark (■) *Tetrix subulata* colour morphs were observed eating each of the five types of food.

colour morphs, but the preference was expressed most strongly by individuals belonging to the dark morph (Fig. 2). The *Mnium* carpet moss was the least used food type by all colour morphs. Separate analyses for each food type revealed that the *Carex* vernal sedge was preferred to a significantly greater extent by individuals belonging to the light morph than by striped and dark morphs ($P < 0.05$, Fig. 2). The three colour morphs did not differ markedly in their utilization of the remaining food types (all $P > 0.05$, Fig. 2).

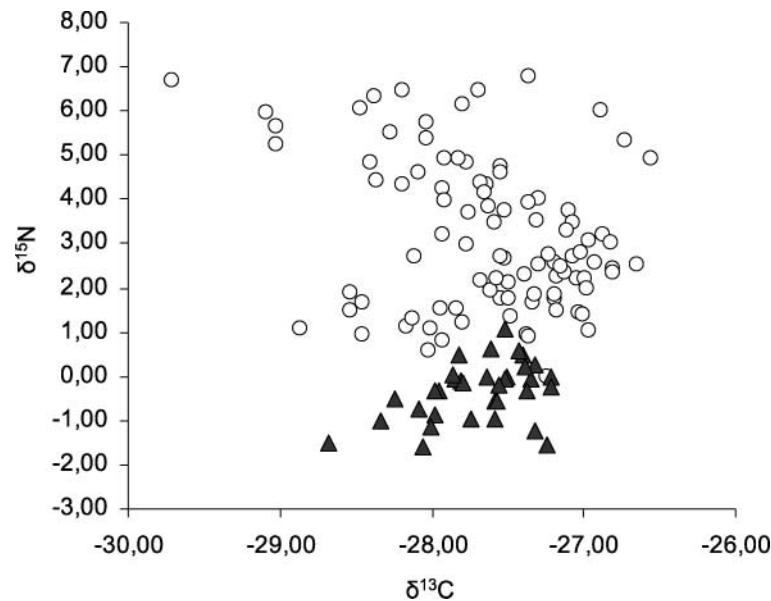
Natural abundance of stable isotopes in relation to source population and colour morph

The mean and range of values of the natural abundance of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for individuals belonging to different colour morphs and originating from the two different populations used in the current experiment are shown in Table 1. Separate estimates obtained from the head and hind femur were highly correlated across individuals for both isotopes in both populations ($\delta^{13}\text{C}$: Vanserum: $r = 0.85$, $n = 91$; Hovmantorp: $r = 0.78$, $n = 34$; $\delta^{15}\text{N}$: Vanserum: $r = 0.95$, $n = 91$; Hovmantorp: $r = 0.75$, $n = 34$; all $P < 0.0001$). Initial exploratory MANOVA indicated significant overall differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between the two populations (Wilks' $\lambda = 0.44$, $F_{1,243} = 153.55$, $P < 0.0001$), between body parts (hind femur vs. head: Wilks' $\lambda = 0.93$, $F_{2,243} = 8.91$, $P = 0.0002$), and between colour morphs (Wilks' $\lambda = 0.95$, $F_{4,486} = 3.47$, $P = 0.0083$) (Table 1). Since we did not have data for all three morphs in both populations, we performed separate tests for differences among colour morphs within each population (Table 1, Fig. 3).

In Vanserum, a MANOVA indicated significant overall differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between body parts (Wilks' $\lambda = 0.87$, $F_{2,180} = 13.46$, $P = 0.0001$) and between colour morphs

Table 1. Natural abundance of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in *Tetrix subulata* representing two populations and three different colour morphs

		$\delta^{13}\text{C}$ (‰) mean (min, max)		$\delta^{15}\text{N}$ (‰) mean (min, max)	
Population/ colour morph	<i>N</i>	Hind femur	Head	Hind femur	Head
Vanserum					
Striped	30	−27.4 (−29.2, −26.0)	−27.9 (−29.4, −27.1)	3.8 (0.6, 6.6)	3.6 (0.7, 6.4)
Light	29	−27.4 (−29.2, −26.4)	−27.9 (−30.2, −27.1)	2.9 (−0.3, 6.9)	2.5 (0.3, 6.4)
Dark	34	−27.4 (−28.3, −26.5)	−27.7 (−28.8, −26.8)	3.2 (0.7, 7.2)	2.9 (−0.2, 6.3)
Hovmantorp					
Striped	17	−27.5 (−28.1, −26.9)	−27.7 (−28.6, −27.2)	−0.2 (−1.6, 0.8)	−0.3 (−1.5, 0.5)
Dark	17	−27.7 (−28.8, −27.1)	−27.9 (−28.6, −27.4)	−0.5 (−2.2, 1.2)	−0.3 (−1.4, 1.0)

**Fig. 3.** Biplot of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope signatures of *Tetrix subulata* females originating from two different natural populations in Vanserum (○) and Hovmantorp (▲). Individual mean values of estimates from head and hind femur are shown.

(Wilks' $\lambda = 0.93$, $F_{4,360} = 3.10$, $P = 0.016$) (Table 1, Fig. 4). Separate two-way ANOVAs on each of the isotopes, and with colour morph and body part as the independent variables, revealed significant differences in $\delta^{15}\text{N}$ between colour morphs ($F_{2,181} = 5.38$, $P = 0.0054$) but not between body parts ($F_{1,181} = 1.24$, $P = 0.27$). In contrast, there were significant differences in $\delta^{13}\text{C}$ between body parts ($F_{1,181} = 20.01$, $P < 0.0001$) but not between colour morphs ($F_{2,181} = 0.31$, $P = 0.73$).

In Hovmantorp, a MANOVA indicated significant overall differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between body parts (Wilks' $\lambda = 0.86$, $F_{2,64} = 5.34$, $P = 0.0071$) but not between colour

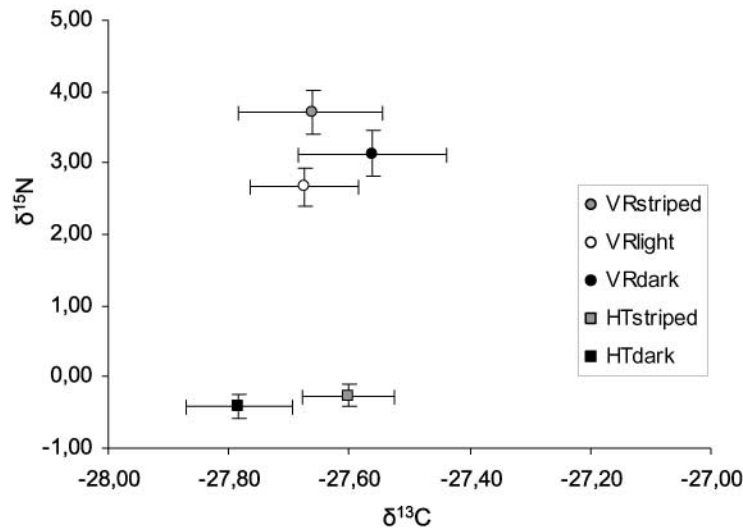


Fig. 4. Biplot of the mean ($\pm$ s.e.) isotope signatures of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for individuals originating from two different natural populations in Vanserum (circles) or Hovmantorp (squares), and belonging to dark (black symbols), striped (grey symbols) or pale (open symbols) colour morphs. Individual mean values of estimates from head and hind femur are shown. Note that scales are different for the vertical and horizontal axes.

morphs (Wilks' $\lambda = 0.94$, $F_{2,64} = 2.16$, $P = 0.12$) (Table 1, Fig. 4). Separate two-way ANOVAs revealed significant differences in $\delta^{13}\text{C}$ both between colour morphs ($F_{1,65} = 4.38$, $P = 0.04$) and between body parts ($F_{1,65} = 8.08$, $P = 0.006$). There were no significant differences in $\delta^{15}\text{N}$ between colour morphs ($F_{1,65} = 0.61$, $P = 0.44$) or body parts ($F_{1,65} = 0.13$, $P = 0.72$).

DISCUSSION

The results of this study provide evidence for dietary divergence among *Tetrix subulata* pygmy grasshoppers that appears to reflect the interplay of differential preferences and ecological constraints. Multiple-choice feeding experiments demonstrated differences in food preferences among individuals originating from different populations (Fig. 1), as well as among individuals that originated from the same population but were different colour morphs: pale, striped or dark (Fig. 2). Analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope signatures further indicated that there were large long-term dietary differences between free-ranging individuals from populations in unlike environments, differences among alternative colour morphs within populations, as well as considerable variation among individuals even within populations and colour morphs (Table 1, Figs. 3 and 4). To our knowledge, this study is the first to indicate differences in both food preferences and long-term realized feeding niches between free-ranging individuals that belong to alternative colour morphs of a generalist species [but see Nosil *et al.* (2006) for a possible example in a phytophagous host-specialist species].

Differences among populations

Inferring dietary differences among populations from isotopic signals is not without caveats because plants of the same species growing in different environments may show different isotopic ratios, and thus base levels of the ecosystems are important (Gannes *et al.*, 1997). In our case, however, both behavioural observations and analyses of isotope signatures indicated dietary differences between populations. Values of $\delta^{13}\text{C}$ were similar, whereas the $\delta^{15}\text{N}$ values differed markedly between individuals that originated from Vanserum or Hovmantorp (Table 1, Fig. 3). The difference in average $\delta^{15}\text{N}$ values between our populations was about 4‰, which exceeds the average 3‰ enrichment seen between different trophic levels in a food web (e.g. Fry *et al.*, 1978; Hyodo *et al.*, 2010). This difference probably reflects the fact that the two populations were exposed to strikingly different environments: a previously forested area that was ravaged by fire the year before capture, versus a stable alkaline fen used as a grazing area for cows (Markow *et al.*, 2000; Asada *et al.*, 2005; Tiunov, 2007; Hyodo *et al.*, 2010; Liu and Wang, 2010).

The lower mean and narrower range of variation of $\delta^{15}\text{N}$ in Hovmantorp compared with Vanserum may in part be attributed to the effect of fire on nitrogen availability (Hogberg, 1997; Grogan *et al.*, 2000). However, instead of the enriched values of $\delta^{15}\text{N}$ expected after fire (Grogan *et al.*, 2000), we found more depleted values in the Hovmantorp population. A possible explanation for this finding is that Tetrigids normally have a broad diet and feed on live plants and partly decomposed organic matter originating from plants and higher trophic levels (Bastow *et al.*, 2002), but their ability to scavenge was limited in Hovmantorp where fire had depleted the amount of litter. An additional explanation is that the main source of food available for grasshoppers in the post-fire environment was mosses. Mosses do not obtain their water and nutrients from the soil via roots but via precipitation from the atmosphere rich in nitrates, which in turn is ^{15}N depleted (Xiao *et al.*, 2010). The relatively narrow range of C and N isotopes in the population in Hovmantorp may also represent an example of niche collapse following an ecological disturbance (Layman *et al.*, 2007).

Differences among alternative colour morphs within populations

Our behavioural observations and analyses of isotope signatures indicated differences in food preferences and long-term dietary habits among individuals that belonged to dark, striped or pale colour morphs *within* populations. The overall differences in isotope signatures among colour morphs were most pronounced in the Vanserum population, where the variation was manifest primarily in $\delta^{15}\text{N}$ (Table 1, Fig. 4). This indicates that colour morphs vary in the extent to which they feed on different plant species, microalgae, and detritus, a conclusion that is corroborated by the morph-specific preferences demonstrated in our behavioural feeding experiment (Fig. 2). The different feeding habits among free-ranging pygmy grasshopper colour morphs probably reflect a combination of dietary preferences and variation in encounter rates with different types of food due to morph-specific differences in microhabitat utilization.

The alternative colour patterns in pygmy grasshoppers are associated with a suite of morphological, physiological, life-history, and behavioural traits (Forsman, 2000; Ahnesjö and Forsman, 2006; Forsman *et al.*, 2011), and it is feasible that the divergence among these eco-morphs includes food preferences (True, 2003; Forsman *et al.*, 2008; McKinnon and Pierotti, 2010). In addition, alternative colour morphs may assimilate similar diets with different efficiencies because

they differ in physiology and body temperature preferences (e.g. Gannes *et al.*, 1997; Yeh and Wang, 2001).

The long-term differences in diet may also in part reflect a by-product of differences in microhabitat selection between alternative colour morphs. Pygmy grasshopper individuals belonging to different colour morphs differentially utilize microhabitats with different biophysical properties to meet demands associated with temperature regulation and predator avoidance behaviours (Forsman and Appelqvist, 1998; Ahnesjö and Forsman, 2006). This may result in differences in isotope signatures among colour morphs, since it has been shown that $\delta^{15}\text{N}$ values may vary among soil types and plants depending on depth of roots, water sources, nitrogen availability in the ecosystem, and the degree of decomposition and humification (Markow *et al.*, 2000; Asada *et al.*, 2005; Tiunov, 2007; Hyodo *et al.*, 2010; Liu and Wang, 2010). Even if the differential microhabitat selection seen in alternative pygmy grasshopper colour morphs has been driven by selection imposed by predation and temperature constraints, this may have resulted in dietary resource specialization by the different colour morphs as a secondary response (Bernays and Graham, 1988).

Studies of other species have shown that more intense competition for resources may lead to increased diet variation between individuals in the same population (e.g. Bolnick, 2001; Svanback and Bolnick, 2007; Tinker *et al.*, 2008). Our findings are at odds with this notion. The larger ranges of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in Vanserum (Table 1, Fig. 2) instead indicate that the greater availability and diversity of plants in Vanserum compared with the recently burned environment in Hovmantorp allowed for a broader diet. Similarly, the lesser diversity of potential food types in Hovmantorp may have limited the scope for resource subdivision, since evidence of long-term dietary differences (as inferred by differences in isotope signatures) among alternative colour morphs were less pronounced in Hovmantorp than in Vanserum.

Inter-individual differences within populations and colour morphs

The range of inter-individual differences in isotope signatures within populations and colour morphs was about 7‰ for $\delta^{15}\text{N}$ and 3‰ for $\delta^{13}\text{C}$ in the Vanserum population (Table 1). This huge range is comparable with the average 6‰ $\delta^{15}\text{N}$ enrichment between three trophic levels (Fry, 1988; Hyodo *et al.*, 2010), and larger than that reported for many other insect species (Taylor *et al.*, 2004; Bourguignon *et al.*, 2009). This may reflect that pygmy grasshoppers include in their diet dead plants and dead animal matter from higher trophic levels (Holst, 1986). However, we cannot infer about trophic levels without knowing the base level of the ecosystem and prey items. The samples used for our isotope analysis were homogeneous; we used adult females only, and individuals from the same population were collected within one week (Vanserum) or on the same day (Hovmantorp) in a small area. Like many other insects (Harrison, 1980), *T. subulata* is wing dimorphic (Rehn and Grant, 1955) and macropterous individuals with fully developed functional wings may resort to active flight, indicating a capacity for long-distance dispersal (Harrison, 1980). However, mark-recapture data of free-ranging individuals indicate that *T. subulata* are relatively sedentary and normally move only a few metres per day (Forsman and Appelqvist, 1999; Caesar *et al.*, 2007). In this context, it is interesting that the $\delta^{15}\text{N}$ range seen in these grasshoppers is comparable to ranges documented for long-lived, wide-roaming predatory mammals, birds, and fishes (Minami *et al.*, 1995; Berglund *et al.*, 2001; Urton and Hobson, 2005; Cherel *et al.*, 2009; Vander Zanden *et al.*, 2010). There are two possible explanations for the large $\delta^{15}\text{N}$ range. Either each individual utilizes a narrow food niche, and different

individuals specialize on different diets (Vander Zanden *et al.*, 2010), or, alternatively, individuals are generalist or opportunist foragers with potentially very broad food niches, and include in their diet different types of food depending on availability (Cherel *et al.*, 2009). The behavioural data from our multiple-choice feeding experiment lend some support to the interpretation that individuals are specialists.

CONCLUSIONS

Based on all the caveats of stable isotopes, Gannes *et al.* (1997) called for a combination of this method with behavioural laboratory experiments to investigate food preferences and dietary habits. Indeed, interpretation of our isotopic data for free-ranging *Tetrix subulata* pygmy grasshopper individuals from different natural populations is facilitated by the results from our multiple-choice feeding experiment; the two approaches not only tell consistent stories, they also point to important interactive roles of colour morph-specific food preferences and constraints for patterns of resource partitioning. Our data on behaviour and isotopic signatures of pale, striped, and dark pygmy grasshoppers provide, to our knowledge, the first evidence of long-term dietary divergence in a colour polymorphic generalist species. The different feeding habits among free-ranging pygmy grasshopper colour morphs indicated by our results (based on isotopic signatures) probably reflect a combination of dietary preferences (as demonstrated by our feeding experiment) on the one hand, and variation in encounter rates with different types of food due to morph-specific differences in microhabitat utilization associated with predator avoidance and temperature regulation on the other. Information on isotopes only does not reveal the causes and possible role of food preferences for dietary divergence. Conversely, laboratory feeding experiments alone do not reveal whether food preferences translate into differential diets among free-ranging individuals in the wild.

That the magnitude of the dietary differences shown in this study is sufficient to be of ecological importance is supported by our previous demonstration that under high-density conditions, average survival is higher in more diverse groups of *T. subulata* where individuals belong to different colour morphs than in more homogeneous groups (Caesar *et al.*, 2010). Differential food utilization by individuals belonging to alternative colour morphs may have played an important role in intra-population divergence, by promoting the evolution of phenotypic integration and 'alternative adaptations' or 'complex phenotypes' by means of covariance between colour pattern and behavioural, physiological, morphological, and life-history traits (Lande and Arnold, 1983; Westerberhard, 1986; Wright, 1988; Arnold *et al.*, 2001). It has been hypothesized that dietary differences among alternative phenotypes may contribute to the increased ecological and evolutionary success of polymorphic populations and species by reducing the intensity of intra-specific competition, and by promoting population stability and persistence, colonization success, and speciation (Bolnick *et al.*, 2003; Forsman *et al.*, 2008; Hughes *et al.*, 2008). Pygmy grasshoppers and other colour polymorphic species offer suitable model systems for testing these contentions in the future.

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REFERENCES

- Ahnesjö, J. and Forsman, A. 2003. Correlated evolution of colour pattern and body size in polymorphic pygmy grasshoppers, *Tetrix undulata*. *J. Evol. Biol.*, **16**: 1308–1318.
- Ahnesjö, J. and Forsman, A. 2006. Differential habitat selection by pygmy grasshopper color morphs: interactive effects of temperature and predator avoidance. *Evol. Ecol.*, **20**: 235–257.
- Anthony, C.D., Venesky, M.D. and Hickerson, C.A.M. 2008. Ecological separation in a polymorphic terrestrial salamander. *J. Anim. Ecol.*, **77**: 646–653.
- Arnold, S.J. 1977. Polymorphism and geographic variation in the feeding behavior of the garter snake *Thamnophis elegans*. *Science*, **197**: 676–678.
- Arnold, S.J., Pfrender, M.E. and Jones, A.G. 2001. The adaptive landscape as a conceptual bridge between micro- and macroevolution. *Genetica*, **112**: 9–32.
- Asada, T., Warner, B.G. and Aravena, R. 2005. Nitrogen isotope signature variability in plant species from open peatland. *Aquat. Bot.*, **82**: 297–307.
- Bastow, J.L., Sabo, J.L., Finlay, J.C. and Power, M.E. 2002. A basal aquatic-terrestrial trophic link in rivers: algal subsidies via shore-dwelling grasshoppers. *Oecologia*, **131**: 261–268.
- Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A. and Macleod, H. 2004. Determining trophic niche width: a novel approach using stable isotope analysis. *J. Anim. Ecol.*, **73**: 1007–1012.
- Berglund, O., Larsson, P. and Broman, D. 2001. Organochlorine accumulation and stable isotope ratios in an Atlantic salmon (*Salmo salar*) population from the Baltic Sea. *Sci. Total Environ.*, **281**: 141–151.
- Bernays, E. and Graham, M. 1988. On the evolution of host specificity in phytophagous arthropods. *Ecology*, **69**: 886–892.
- Bolnick, D.I. 2001. Intraspecific competition favours niche width expansion in *Drosophila melanogaster*. *Nature*, **410**: 463–466.
- Bolnick, D.I., Yang, L.H., Fordyce, J.A., Davis, J.M. and Svanback, R. 2002. Measuring individual-level resource specialization. *Ecology*, **83**: 2936–2941.
- Bolnick, D.I., Svanback, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Hulsey, C.D. *et al.* 2003. The ecology of individuals: incidence and implications of individual specialization. *Am. Nat.*, **161**: 1–28.
- Bond, A.B. 2007. The evolution of color polymorphism: crypticity searching images, and apostatic selection. *Annu. Rev. Ecol. Syst.*, **38**: 489–514.
- Bourguignon, T., Sobotnik, J., Lepoint, G., Martin, J.-M. and Roisin, Y. 2009. Niche differentiation among neotropical soldierless soil-feeding termites revealed by stable isotope ratios. *Soil Biol. Biochem.*, **41**: 2038–2043.
- Caesar, S., Ahnesjö, J. and Forsman, A. 2007. Testing the role of coadapted genes versus bet-hedging for mating strategies in colour polymorphic pygmy grasshoppers. *Biol. J. Linn. Soc.*, **90**: 491–499.
- Caesar, S., Karlsson, M. and Forsman, A. 2010. Diversity and relatedness enhance survival in colour polymorphic grasshoppers. *PLoS One*, **5**: e10880.
- Cherel, Y., Kernalguyen, L., Richard, P. and Guinet, C. 2009. Whisker isotopic signature depicts migration patterns and multi-year intra- and inter-individual foraging strategies in fur seals. *Biol. Lett.*, **5**: 830–832.
- Deniro, M.J. and Epstein, S. 1978a. Carbon isotopic evidence for different feeding patterns in two Hyrax species occupying the same habitat. *Science*, **201**: 906–908.
- Deniro, M.J. and Epstein, S. 1978b. Influence of diet on distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta*, **42**: 495–506.
- Elmer, K.R., Lehtonen, T.K., Kautt, A.F., Harrod, C. and Meyer, A. 2010. Rapid sympatric ecological differentiation of crater lake cichlid fishes within historic times. *BMC Biol.*, **8**: 60.

- Endler, J.A. 1983. Natural and sexual selection on color patterns in poeciliid fishes. *Environ. Biol. Fish.*, **9**: 173–190.
- Forsman, A. 1997. Thermal capacity of different colour morphs in the pygmy grasshopper *Tetrix subulata*. *Ann. Zool. Fenn.*, **34**: 145–149.
- Forsman, A. 2000. Some like it hot: intra-population variation in behavioral thermoregulation in color-polymorphic pygmy grasshoppers. *Evol. Ecol.*, **14**: 25–38.
- Forsman, A. and Appelqvist, S. 1998. Visual predators impose correlational selection on prey color pattern and behavior. *Behav. Ecol.*, **9**: 409–413.
- Forsman, A. and Appelqvist, S. 1999. Experimental manipulation reveals differential effects of colour pattern on survival in male and female pygmy grasshoppers. *J. Evol. Biol.*, **12**: 391–401.
- Forsman, A., Ahnesjö, J., Caesar, S. and Karlsson, M. 2008. A model of ecological and evolutionary consequences of color polymorphism. *Ecology*, **89**: 34–40.
- Forsman, A., Karlsson, M., Wennersten, L., Johansson, J. and Lapid, E. 2011. Rapid evolution of fire melanism in replicated populations of pygmy grasshoppers. *Evolution*, **65**: 2530–2540.
- Fry, B. 1988. Food web structure on Georges bank from stable C, N and S isotopic compositions. *Limnol. Oceanogr.*, **33**: 1182–1190.
- Fry, B., Joern, A. and Parker, P.L. 1978. Grasshopper food web analysis – use of carbon isotope ratios to examine feeding relationships among terrestrial herbivores. *Ecology*, **59**: 498–506.
- Futuyma, D.J. and Peterson, S.C. 1985. Genetic variation in the use of resources by insects. *Annu. Rev. Entomol.*, **30**: 217–238.
- Gannes, L.Z., Obrien, D.M. and delRio, C.M. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. *Ecology*, **78**: 1271–1276.
- Green, M.C. 2005. Plumage dimorphism in the reddish egret: does plumage coloration influence foraging habitat use and tactics? *Waterbirds*, **28**: 519–524.
- Grogan, P., Bruns, T.D. and Chapin, F.S. 2000. Fire effects on ecosystem nitrogen cycling in a Californian bishop pine forest. *Oecologia*, **122**: 537–544.
- Harrison, R.G. 1980. Dispersal polymorphisms in insects. *Annu. Rev. Ecol. Syst.*, **11**: 95–118.
- Hochkirch, A., Groning, J., Loos, T., Metzger, C. and Reichelt, M. 2000. Specialized diet and feeding habits as key factors for the habitat requirements of the grasshopper species *Tetrix subulata* (Orthoptera: Tetrigidae). *Entomol. Gen.*, **25**: 39–51.
- Hogberg, P. 1997. Tansley review No. 95. ¹⁵N natural abundance in soil–plant systems. *New Phytol.*, **137**: 179–203.
- Holst, K.T. 1986. The Saltatoria of northern Europe. *Fauna Entomol. Scand.*, **16**: 1–127.
- Hood-Nowotny, R. and Knols, B.G.J. 2007. Stable isotope methods in biological and ecological studies of arthropods. *Entomol. Exp. Appl.*, **124**: 3–16.
- Hughes, A.R., Inouye, B.D., Johnson, M.T.J., Underwood, N. and Vellend, M. 2008. Ecological consequences of genetic diversity. *Ecol. Lett.*, **11**: 609–623.
- Hyodo, F., Kohzu, A. and Tayasu, I. 2010. Linking aboveground and belowground food webs through carbon and nitrogen stable isotope analyses. *Ecol. Res.*, **25**: 745–756.
- Jones, J.S. 1982. Genetic differences in individual behavior associated with shell polymorphism in the snail *Cepaea nemoralis*. *Nature*, **298**: 749–750.
- Karlsson, M., Caesar, S., Ahnesjö, J. and Forsman, A. 2008. Dynamics of colour polymorphism in a changing environment: fire melanism and then what? *Oecologia*, **154**: 715–724.
- Karlsson, M., Johansson, J., Caesar, S. and Forsman, A. 2009. No evidence for developmental plasticity of color patterns in response to rearing substrate in pygmy grasshoppers. *Can. J. Zool.*, **87**: 1044–1051.
- Lande, R. and Arnold, S.J. 1983. The measurement of selection on correlated characters. *Evolution*, **37**: 1210–1226.
- Layman, C.A., Quattrochi, J.P., Peyer, C.M. and Allgeier, J.E. 2007. Niche width collapse in a resilient top predator following ecosystem fragmentation. *Ecol. Lett.*, **10**: 937–944.

- Liu, X.J. and Wang, G. 2010. Measurements of nitrogen isotope composition of plants and surface soils along the altitudinal transect of the eastern slope of Mount Gongga in southwest China. *Rapid Commun. Mass. SP*, **24**: 3063–3071.
- Markow, T.A., Anwar, S. and Pfeiler, E. 2000. Stable isotope ratios of carbon and nitrogen in natural populations of *Drosophila* species and their hosts. *Funct. Ecol.*, **14**: 261–266.
- McKinnon, J.S. and Pierotti, M.E.R. 2010. Colour polymorphism and correlated characters: genetic mechanisms and evolution. *Mol. Ecol.*, **19**: 5101–5125.
- Merilaita, S. and Jormalainen, V. 1997. Evolution of sex differences in microhabitat choice and colour polymorphism in *Idotea baltica*. *Anim. Behav.*, **54**: 769–778.
- Minami, H., Minagawa, M. and Ogi, H. 1995. Changes in stable carbon and nitrogen isotope ratios in sooty and short-tailed shearwaters during their northward migration. *Condor*, **97**: 565–574.
- Morey, S.R. 1990. Microhabitat selection and predation in the pacific treefrog, *Pseudacris regilla*. *J. Herpetol.*, **24**: 292–296.
- Nabours, R.K. 1929. The genetics of the Tettigidae (Grouse locusts). *Bibliographia Genetica Verlag M Nijho Den Haag, Netherlands*, 27–104.
- Nosil, P., Sandoval, C.P. and Crespi, B.J. 2006. The evolution of host preference in allopatric vs. parapatric populations of *Timema cristinae* walking-sticks. *J. Evol. Biol.*, **19**: 929–942.
- Polis, G.A. 1984. Age structure component of niche width and intraspecific resource partitioning – can age-groups function as ecological species. *Am. Nat.*, **123**: 541–564.
- Pulliam, H.R. 1974. Theory of optimal diets. *Am. Nat.*, **108**: 59–74.
- Rehn, J.A.G. and Grant, H.J.J. 1955. *Tetrix subulata* (Orthoptera; Acridoidea; Tettigidae) as occurring in North America. *Proc. Acad. Nat. Sci. Philadelphia*, **107**: 145–165.
- Roughgarden, J. 1972. Evolution of niche width. *Am. Nat.*, **106**: 683–718.
- SAS. 2004. *Help and Documentation*. Cary, NC: SAS Institute.
- Shine, R., Ambariyanto, Harlow, P.S. and Mumpuni. 1998. Ecological divergence among sympatric colour morphs in blood pythons, *Python brongersmai*. *Oecologia*, **116**: 113–119.
- Shine, R., Shine, T. and Shine, B. 2003. Intraspecific habitat partitioning by the sea snake *Emydocephalus annulatus* (Serpentes, Hydrophiidae): the effects of sex, body size, and colour pattern. *Biol. J. Linn. Soc.*, **80**: 1–10.
- Skulason, S. and Smith, T.B. 1995. Resource polymorphisms in vertebrates. *Trends Ecol. Evol.*, **10**: 366–370.
- Smith, T.B. and Skulason, S. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. *Annu. Rev. Ecol. Syst.*, **27**: 111–133.
- Sokal, R.R. and Rohlf, F.J. 1981. *Biometry: The Principles and Practice of Statistics in Biological Research*, 2nd edn. San Francisco, CA: W.H. Freeman.
- Stephens, D.W. and Krebs, J.R. 1986. *Foraging Theory*. Monographs in Behavior and Ecology. Princeton, NJ: Princeton University Press.
- Svanback, R. and Bolnick, D.I. 2007. Intraspecific competition drives increased resource use diversity within a natural population. *Proc. R. Soc. Lond. B*, **274**: 839–844.
- Taylor, S.J., Hackley, K., Krejca, J.K., Dreslik, M.J., Greenberg, S.E. and Raboin, E.L. 2004. Examining the role of cave crickets (Rhaphidophoridae) in Central Texas cave ecosystems: isotope ratios ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) and radio tracking. *Illinois Natural History Survey Center for Biodiversity Technical Report*, No. 9: 1–128.
- Tinker, M.T., Bentall, G. and Estes, J.A. 2008. Food limitation leads to behavioral diversification and dietary specialization in sea otters. *Proc. Natl. Acad. Sci. USA*, **105**: 560–565.
- Tiunov, A.V. 2007. Stable isotopes of carbon and nitrogen in soil ecological studies. *Biol. Bull.*, **34**: 395–407.
- Todd, P.A., Briers, R.A., Ladle, R.J. and Middleton, F. 2006. Phenotype–environment matching in the shore crab (*Carcinus maenas*). *Mar. Biol.*, **148**: 1357–1367.
- True, J.R. 2003. Insect melanism: the molecules matter. *Trends Ecol. Evol.*, **18**: 640–647.

- Urton, E.J.M. and Hobson, K.A. 2005. Intrapopulation variation in gray wolf isotope (δ N-15 and δ C-13) profiles: implications for the ecology of individuals. *Oecologia*, **145**: 317–326.
- Vander Zanden, H.B., Bjorndal, K.A., Reich, K.J. and Bolten, A.B. 2010. Individual specialists in a generalist population: results from a long-term stable isotope series. *Biol. Lett.*, **6**: 711–714.
- Westerberhard, M.J. 1986. Alternative adaptations, speciation, and phylogeny (a review). *Proc. Natl. Acad. Sci. USA*, **83**: 1388–1392.
- Wilson, G.C. and Jamieson, I.G. 2005. Does melanism influence the diet of the mountain stone weta *Hemideina maori* (Orthoptera: Anostomatidae)? *NZ J. Ecol.*, **29**: 149–152.
- Wimberger, P.H. 1994. Trophic polymorphisms, plasticity, and speciation in vertebrates. In *Theory and Application in Fish Feeding Ecology* (D.J. Stouder, K.L. Fresh and R.J. Feller, eds.), pp. 19–43. Columbia, SC: University of South Carolina Press.
- Wright, S. 1988. Surfaces of selective value revisited. *Am. Nat.*, **131**: 115–123.
- Xiao, H.-Y., Tang, C.-G., Xiao, H.-W., Liu, X.-Y. and Liu, C.-Q. 2010. Stable sulphur and nitrogen isotopes of the moss *Haplocladium microphyllum* at urban, rural and forested sites. *Atmospheric Environ.*, **44**: 4312–4317.
- Yeh, H.-W. and Wang, W.-M. 2001. Factors affecting the isotopic composition of organic matter. (1) Carbon isotopic composition of terrestrial plant materials. *Proc. Natl. Sci. CR China*, **25**: 137–147.

