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Ecotypic differentiation in the grasshopper *Chorthippus brunneus*: life history varies in relation to climate

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Abstract Life history variations among 27 populations of the grasshopper *Chorthippus brunneus* from around the British Isles were examined under laboratory conditions over three generations. Multiple-regression analysis was used to examine the relationship between grasshopper life histories and the climates of their ancestral sites. Grasshoppers from cooler sites were heavier at hatching. Grasshoppers from northern sites grew faster and developed through fewer instars, attaining adulthood earlier, at the expense of adult size. Depending on the measure of adult size used, adults were larger in warmer, sunnier or more southerly locations. Ecotypic differentiation is probably widespread among animals as it is among plants, though it is more rarely demonstrated by zoological studies, especially over the wide geographical scale covered here. Evidence from regression analysis supports the hypothesis that ecotypic differentiation in *C. brunneus* is an evolutionary response to climatic variation. The existence of intraspecific genetic diversity for climatic adaptations has implications for biodiversity conservation and the understanding of biotic responses to climatic change. It deserves wider recognition.

Key words Ecotypes · Geographic variation · Intraspecific variation · Orthoptera · Acrididae

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Introduction

Most of the world's species are geographically widespread and thus inhabit a wide range of environments. A population in one place may experience different selection pressures from populations elsewhere. Where gene flow between such populations is sufficiently low, natural selection may create genetic differences between them (Stearns 1992). Turesson (1922) coined the term 'ecotype' to describe apparently adaptive genetic differences between populations of plant species (Begon et al. 1996). When individuals from such populations are grown together under the same environmental conditions, any differences between populations may be ascribed to a genetic rather than environmentally induced origin. 'Ecotypic differentiation' may be said to have occurred.

There have been many studies of ecotypic differentiation in the plant kingdom. Populations have been compared from different altitudes (e.g. Clausen et al. 1940, 1948; Mariko and Koizumi 1993; Bennington and McGraw 1995), different geographical regions, especially different latitudes (e.g. Robertson and Ward 1970; Potvin 1986; Sawada et al. 1994; Norton et al. 1995) or along other environmental gradients such as concentrations of heavy metals (Antonovics and Bradshaw 1970), or sea temperatures (Bruhn and Gerard 1996). A wide range of traits has been studied and ascribed to geographical variation in a wide range of environmental variables.

Comparable studies in the animal kingdom are relatively few, perhaps because the geographical variation in morphology and phenology of animals is less striking. In addition, comparative culture, and reciprocal transplant experiments are more easily carried out with plants than with animals. In their review, Clausen and Hiesey (1958) described only six animal species, or species-groups, in which ecotypic differences had been investigated. They comprised one mammal (the deer mouse *Peromyscus maniculatus* and allies), two poikilothermic vertebrates (the stickleback *Gasterosteus aculeatus*, and the frog

Rana pipiens) and three insects (*Colias* butterflies, the gypsy moth *Lymantria dispar*, and *Drosophila*).

Studies on animals which do address geographical variation in phenotypes frequently do not differentiate between environmentally induced and genetic components (e.g. Bradshaw 1974; Gill 1979; Sota 1986; Nylin and Svärd 1991; and examples in the review by Ray 1960). Those studies that use a 'common garden' laboratory rearing programme can distinguish genetic differences between putative ecotypes (e.g. Strong 1972; Berven et al. 1979; Berven and Gill 1983; Mousseau and Roff 1989; Ayres and Scriber 1994; and references in Mousseau 1997 and Partridge and Coyne 1997). The genotypic effect may be very different, even opposite, to observed clines in the field (Berven et al. 1979).

Some studies have examined geographical variation in genetic traits in relation to environmental variation. Of the two *Gpdh* allozymes, *S* and *F*, in *Drosophila*, the *S* allele is more frequent at higher latitudes. Barnes et al. (1989) found a significant allozyme × temperature interaction for viability among *Drosophila* cultures from three continents. Hewitt and John (1970) found that the frequency of B chromosomes in the grasshopper *Myrmeleotettix maculatus* varied throughout Britain and was strongly correlated with climatic conditions. In a more detailed study of this system at a Welsh site, Hewitt and Ruscoe (1971) found a steep and stable cline in B chromosome frequency corresponding to a microclimatic temperature gradient. B chromosomes may contain genes which slow development in *M. maculatus*; they are more frequent in warmer macro- and microclimates where delayed maturity would be expected to be more advantageous.

Zoologists appear to lack a standard terminology for describing these phenomena. Though ecotypic differentiation was originally described in plants, it is not a term which should be restricted to botanical studies.

Geographical variation in grasshopper phenotypes

Several studies have shown inter-population phenotypic variation for *Chorthippus brunneus* (Thunberg) and other Orthoptera (Mousseau 1997 and references therein). Richards and Waloff (1954) found different proportions of colour varieties in the field in populations separated by a quarter of a mile (0.16 km) at most. Sibly and Monk (1987) found life history differences (in egg size and nymphal development period) in field populations separated by up to 8 km (Monk 1985). Atkinson and Begon (1987a, 1987b) found differences in *M. maculatus* egg sizes, clutch sizes and adult sizes between three populations separated by several kilometres. Thorens (1991) found that the proportion of individuals of *C. mollis* including an additional instar in their development varied between two sites in the Swiss Jura. All these traits are phenotypically plastic (Richards and Waloff 1954; Grayson 1984; Hassall and Grayson 1987; Willott 1992); the occurrence of phenotypic variation between populations does not prove the presence of underlying genetic variation.

A genetic basis to inter-population variation in life history has been demonstrated by a few studies on grasshoppers and crickets using common garden rearing. Mousseau and Roff (1989) found latitudinal trends in body size, voltinism and diapause expression in a North American cricket *Allonemobius fasciatus* after at least two laboratory generations. Similar results have been found by Dingle et al. (1990) and Masaki (1967) over equally large distances. Working on a British grasshopper, Atkinson and Begon (1987b) found genetic differences between populations separated by only a few kilometres.

The life history of *C. brunneus*

C. brunneus, the field grasshopper, has an annual life cycle, overwintering exclusively in the egg stage (Richards and Waloff 1954). Hatchlings typically pass through four nymphal instars before the final eclosion into the adult stage. Some females insert an additional instar, 'IIa', into their development (Hassall and Grayson 1987). Sexual maturity is reached a few days after final eclosion. Females are iteroparous, laying batches of on average ten eggs in pods in the soil (Richards and Waloff 1954). The earliest hatchlings may be observed in the field in late April, with adults surviving into late autumn if weather conditions permit. *C. brunneus* feeds on grasses, inhabiting a broad range of grasslands, with a preference for drier sites, shorter swards and sunnier aspects (Richards and Waloff 1954; Marshall and Haes 1990; Haes and Harding 1997).

Climatic effects on life history variables

Climate is the major axis of environmental variation at large geographical scales. It wields an inescapable and important influence on the environment of insects (Uvarov 1931). Most authors have thus sought to explain their observations of geographical variation as adaptations to climatic variation.

Body temperature is vitally important to grasshoppers (Hill and Taylor 1933; Willott 1992; Willott and Hassall 1998) and yet the British range of *C. brunneus* extends the length and breadth of the country (Haes and Harding 1997), covering a wide climatic range. We hypothesise that gross climatic variation between sites is a major correlate of inter-population variation, and advance hypotheses for the possible adaptive significance of relationships between climate and individual components of the grasshopper life history strategy.

Hatchling size

Larger hatchlings are widely thought to hatch from larger eggs (e.g. Monk 1985; Cherrill and Begon 1991). Egg size is assumed to be proportional to the amount of provisions for the embryo. Furthermore, it is assumed that greater resources confer upon the hatchlings better resilience to cold and to poor feeding conditions. Thus larger hatchlings are expected where spring temperatures are cooler or duller.

A subsidiary hypothesis relating to hatchling weights may also be proposed: climatic variables which are significantly related to adult female size would also be related to hatchling size, since larger females may lay larger eggs (Atkinson and Begon 1987a).

Juvenile growth and development rates, and adult size

Larger females have a faster rate of reproductive investment (Richards and Waloff 1954; Willott 1992). Growth rates are thus expected to be nearly maximised, and not subject to trade-offs with other demands on resources. However, attaining larger size at maturity entails a longer juvenile development period. As an annual, univoltine insect, *C. brunneus* may have a limited season within which to complete development and reproduction. Season length may be a key environmental influence on grasshopper life histories (Monk 1985; Sibly and Monk 1987). Alternatively, the increased juvenile mortality incurred during a longer juvenile development period may be of key importance (Grant et al. 1993). In either scenario, individuals may face a trade-off between earlier maturation followed by slower reproduction or later maturation and faster reproduction. It is thus hypothesised that there will be selection for longer juvenile development periods and larger adults in less harsh environments where juvenile mortality rate is lower or in environments with a longer growth season.

Larger adult size may be favoured by climatic conditions which permit delayed maturation. However, it may also be favoured by climatic conditions which permit faster growth. Favourable growth conditions for grasshoppers are provided by warm ambient temperatures, but more importantly by bright sunshine which allows grasshoppers to raise their body temperatures by basking.

Materials and methods

Culture material and methods

Collecting details (1986)

During August and September 1986, 27 collections of grasshoppers were made from sites in the British Isles (Fig. 1), and cultures were founded in the laboratory in Norwich. Collecting sites were selected to cover a wide geographical and climatic range. Most sites selected were within a few kilometres of a meteorological station contributing official observer's returns to the UK Meteorological Office. At each site, usually 20 adult grasshoppers (12 females, 8 males) were collected from a restricted area, representing a single colony. These founders were returned to the laboratory in Norwich and established in standard rearing conditions within a few days of collection.

From two of the sites, two cultures were collected. In each case, the cultures were collected from discrete populations a kilometre or more apart. Although these populations might be expected to be more closely related genetically than more widely separated populations, in fact substantial life history differences were detected, in keeping with the evidence from previous fine-scale studies in grasshoppers (e.g. Atkinson and Begon 1987b). In such cases, both sites have been included in each analysis.

Rearing conditions (1986–1989)

Grasshoppers were reared through three filial laboratory generations (F_1, F_2 and F_3) over 3 years (1987–1989). In each year, hatching was staggered over the length of the summer at intervals of a few days to spread the workload, the cultures being hatched in the same sequence each year.

Cultures were set up in the laboratory in glass-fronted aluminium cages (32.5 × 32.5 × 41 cm high) at a constant background

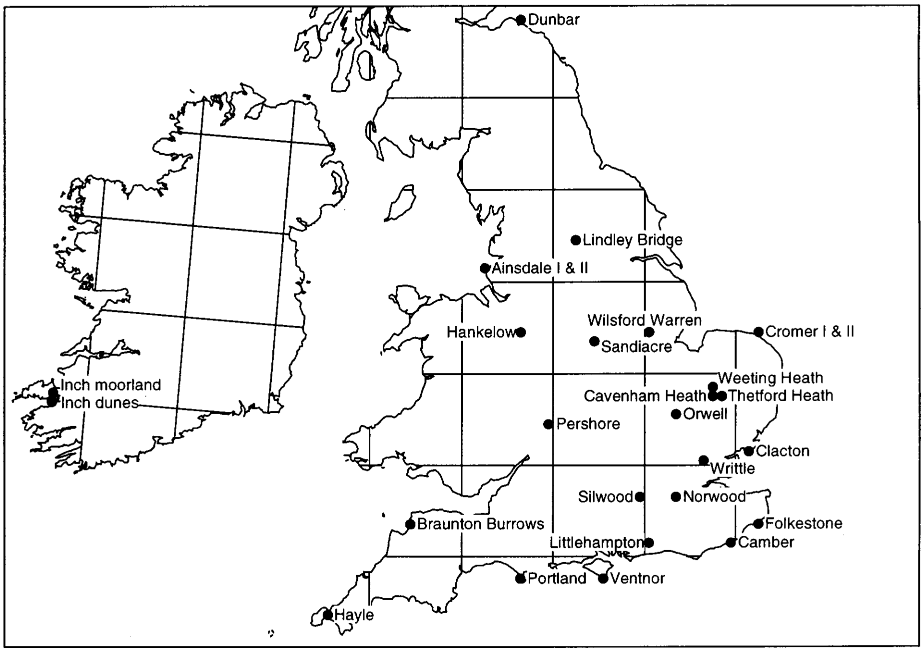

Fig. 1 Sites from which grasshoppers were collected

temperature of 25°C. Light and additional heat were provided for 14 h a day by a 40-W tungsten-filament bulb in the internal light socket of each cage. Oviposition sites were provided in each cage in the form of a small cylindrical perspex pot (50 mm diameter, 40 mm depth) containing dry, sterile sand.

An unlimiting supply of fresh grass was provided as food. A mixture of *Poa pratensis*, *Agrostis capillaris* and *Festuca ovina* was used to feed grasshoppers from 6 weeks after sowing.

Treatment of egg pods and hatchlings

Egg pods were sieved out of the oviposition pots every few days and transferred to small air-tight pots full of 10% (volume by weight) moist sterile sand. Typically, six egg pods were placed in each pot. These pots were kept under laboratory conditions for approximately 3 weeks (long enough to reach the diapause stage; Cherrill and Begon 1991), before being transferred to constant-temperature dark conditions at 4°C to break diapause.

After overwintering, egg pods were removed from cold storage and transferred to a growth cabinet with a 12:12 light:dark cycle, 30°C day temperature (0800–2000 hours) and 20°C night temperature (2000–0800 hours). Prior to hatching, egg pods were repotted to large cylindrical perspex pots (55 mm diameter, 60 mm depth), half-filled with 10% moist sterile sand and containing a disc of filter paper inside the tight-fitting polythene lid (to prevent drops of condensation forming which can be fatal to hatchling grasshoppers).

For each population, between eight and ten pods were removed from cold storage for hatching. Cultures in each successive generation were started with 20 males and 25 females where possible. These hatchlings were placed in aluminium cages as described above.

Data collection: hatchlings

In the F_1 generation, hatchlings were sexed, and up to 20 of each sex were weighed individually. In subsequent generations, hatchlings were not sexed before weighing. In the F_2 generation only, data on pod size (number of eggs per pod) and egg viability were gathered. For each population, hatchling weight data come from up to ten families.

Data collection: the IIa instar in 1987

During the first few days of nymphal life in the F_1 generation, all individuals of a culture were kept together in a single cage. Females were removed from the cage following their first ecdysis (from instar I to instar II) and transferred singly to individual cages, still at 25°C with a 40-W basking bulb. For most populations, all the surviving females in a culture were treated in this way. For each individual female, the date of its next ecdysis was recorded, and the next instar (either IIa or III) was identified. The individual was then weighed, and returned to the main cage.

Data collection: adult eclosion

In the F_1 generation all animals, of all populations and both sexes, were weighed soon after final eclosion (into the adult stage), and the date of adult eclosion was recorded. In the F_3 generation, only females were weighed at adult eclosion. For each sex, and all populations, mean nymphal development periods were estimated as the difference between mean eclosion date and mean hatching date. For each sex, and all populations, mean instantaneous relative growth rates (RGRs, Eq. 1) were calculated, assuming exponential growth from hatching to adult eclosion.

$$\mathrm{RGR} = \frac{\ln(\text{adult eclosion weight}) - \ln(\text{hatchling weight})}{\text{nymphal development period}} \quad (1)$$

Data collection: mature animals

During early mature life, all surviving F_1 generation females and males were weighed (mature weight) on a single date, 1 week after the last female matured. In later mature life, 1 week after the first mature weighing, all surviving females and males were again weighed (final weight), all on the same date. Hind femur lengths were also measured on this date. Mature and final weights are near-equivalent measures. Mature females were weighed on two occasions only because their weights fluctuate greatly between oviposition events. For each of the populations in the F_2 generation, all adult females were weighed on a single date, once all had become adult.

Climate data

The UK Meteorological Office 30-year climate averages for 1961–1990 were used; the collection of these data is described in Meteorological Office (1982). For each grasshopper collecting site, the nearest meteorological station was selected. The distance between grasshopper collecting site and meteorological station varied up to 30 km (mean = 8.1 km, SD = 6.4, n = 25). For five sites there was no station returning sunshine data within an acceptable distance. No meteorological data were available for the Irish collecting site. Three climatic variables were chosen for analysis: mean of monthly means of 24-h maximum temperatures (°C, from 0900 hours GMT), mean of monthly means of 24-h minimum temperatures (°C, from 0900 hours GMT) and mean of monthly means of sunshine amount (h, 24 h from 2400 hours GMT). As well as a mean for each month at each site, an annual mean at each site was used. Maximum temperatures are assumed to relate to daytime temperatures in their biological interpretation, and minimum temperatures to nocturnal temperatures. Further, it is assumed that these air temperatures at 1.25 m above ground level (Meteorological Office 1982) are reasonable predictors of temperatures at grasshopper level, i.e. in the sward (adults and nymphs), or within the top few centimetres of the soil (eggs).

Results

Detection of and correction for uncontrolled sequential changes in rearing conditions

The sequence variable (the order in which the populations were reared in the laboratory) has been correlated with all the life history variables to check for the existence of sequence effects. Sequence effects indicate uncontrolled changes in laboratory conditions during the course of the study.

In analysing sequence effects, data from 28 cultures have been used. The 28th and final culture reared in each year was a replicate of the first population in the sequence: Lindley Bridge. Egg pods laid by the founders in 1986 were divided at random to form these two separate cultures in 1987 and subsequently. They provided a check on the presence of sequence effects.

The sequence variable was found to be significantly correlated with several of the life history variables. Significant positive correlations were found between rearing sequence and nymphal development periods of both males and females (males: $r = 0.5843$, $n = 28$, $P = 0.001$; females: $r = 0.6942$, $n = 28$, $P < 0.0005$). For females alone, significant positive correlations were found between rearing sequence and duration of the first instar ($r = 0.6546$, $n = 25$, $P < 0.0005$) and duration of the second instar ($r = 0.4236$, $n = 22$, $P = 0.049$). For both sexes, the strongest correlation obtained was a negative one with instantaneous relative growth rate to adult eclosion (males: $r = -0.6127$, $n = 28$, $P = 0.001$; females: $r = -0.7531$, $n = 28$, $P < 0.0005$).

These highly significant correlations may be explained as the result of seasonal variation in greenhouse conditions affecting the grass grown to feed the grasshoppers. This variation is reflected in the reduced performance (indicated by growth rate) of grasshoppers at later dates. These uncontrolled effects on the grasshoppers can be adequately compensated for by statistical correction techniques such as partial correlation and partial regression. Such corrections have been applied throughout the analyses described below.

Regressions of life history data on climate data

Analysis

The stepwise method was used to select a multiple linear regression model for each of 28 life history variables regressed against 34 independent variables: maximum and minimum temperatures (for each month and annually), sunshine (for May to September and annually), national grid northing and easting. Rearing sequence was entered first in all models. Stepwise regression constructs a model by sequentially selecting the variable with the highest partial regression coefficient and checking it against the entry and exit criteria. The process stops when no more variables satisfy the criteria. The entry criterion was P (probability of F-to-enter) ≤ 0.05, and the exit criterion $P > 0.10$.

The validity of the assumptions of multivariate normal distributions were examined by checking the normality and homoscedasticity of the distributions of standardised residuals. Standardised residuals were plotted against the sequence variable to check whether time, or the order of data gathering affected the error. Outliers and highly influential points were checked for by calculating Mahalanobis' distance, Cook's distance and other influence measures. Multicollinearity diagnostics were applied to all multivariate models.

Results

Linear multiple-regression models were fitted to the data for 17 of the 28 life history variables measured. However, meteorological variables are highly inter-correlated. In each case where two predictor variables were added to the model (in addition to rearing sequence), multicollinearity diagnostics revealed them to be near dependent upon one another; one was deleted.

For females (and unsexed hatchlings), twelve statistically significant ($P < 0.05$) partial regressions were found. Five life history variables were significantly related only to the national grid northing (which increases to the north, approximating closely to latitude) of the collecting site, after controlling for effects of rearing sequence (Table 1, Figs. 3, 4 and 5). The remaining seven significant partial regressions of life history variables were against meteorological variables (Table 2, Figs. 2, 6).

For males, data were available for eight life history variables. Of those eight, significant partial regressions were found for five (all with climate data). In each case, only a single additional predictor variable was added to the model (Table 3).

Hatchling weights were greater for populations from cooler sites. For F_1 generation hatchlings, the annual mean of maximum temperatures was the best predictor (for both males and females, there being virtually no sex difference in hatchling weight) (Fig. 2), whereas for F_3 hatchlings it was August maximum temperatures. Since August and annual mean maximum temperatures are strongly correlated ($r = 0.8508$, $n = 26$, $P < 0.0005$), these two variables can be considered virtually synonymous.

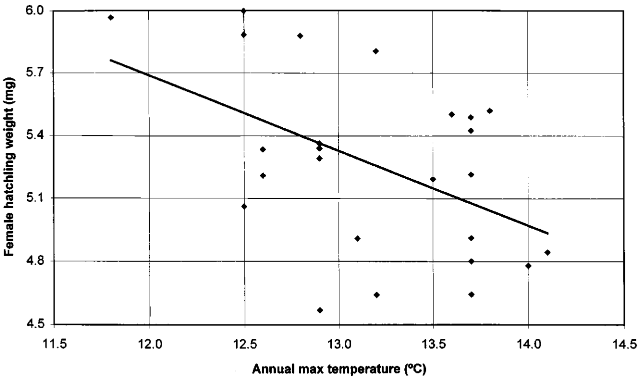

Fig. 2 Hatchlings from warmer sites are lighter, as shown by the regression of female hatchling weight (mean + residual after regression against sequence) against annual maximum temperature. See Table 2 for regression statistics

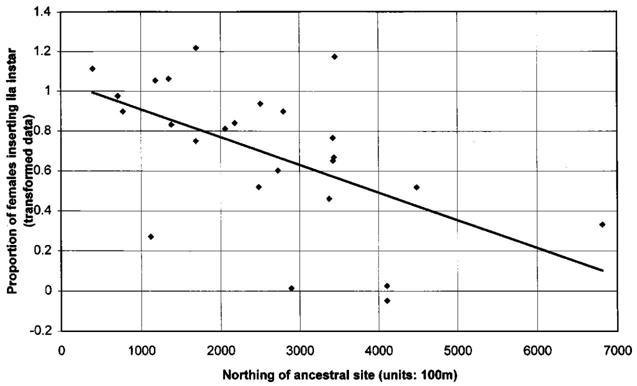

Fig. 3 The IIa instar is less frequent in the north, as shown by the regression of the proportion of females inserting the IIa instar (mean + residuals after regression against sequence) against northing of ancestral site. See Table 1 for regression statistics

Under this laboratory regime, the survival of eggs to hatching (egg viability) was greatest for populations from sites with hot summers (positive correlation with June mean maximum temperatures).

Northern grasshoppers were heavier after two moults, and inserted the IIa instar less frequently at this stage of development (Fig. 3). The development period from hatching to adult eclosion was shorter in the north (Fig. 4), and despite faster nymphal growth (Fig. 5), adults were smaller.

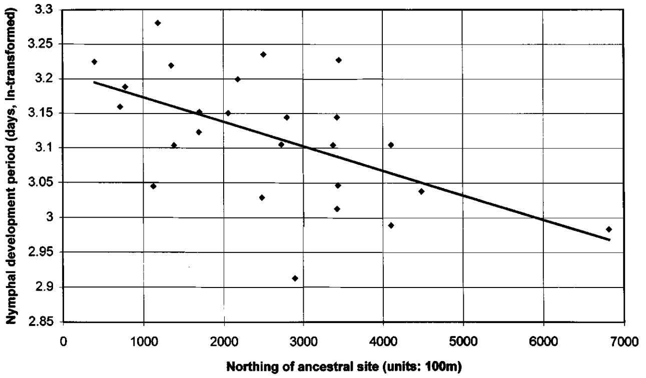

Fig. 4 Nymphal development is faster in the north, as shown by the regression of nymphal development period (mean + residual after regression against sequence) against northing of ancestral site. See Table 1 for regression statistics

Measures of adult size and mature size at different ages and in different generations were correlated with a rather wide range of environmental variables. F_1 generation female eclosion weights were positively correlated with January mean minimum temperatures (Fig. 6); heavier adult females are found in areas of mild winter weather. Similarly, heavier mature females, with longer hind femora, occur where winters are mild, but the correlate for these variables is December mean maximum temperature. Female final weights were correlated more significantly with northing than any of the meteorological variables; again, final weights are greater in the south.

A different set of climatic correlations were exhibited by males. Eclosion weights and mature weights were positively correlated with sunshine levels in June and September, respectively. Final weights, however, were greater where spring minimum temperatures are warmer (positive regression with April mean minimum temperature).

The regression of female adult eclosion weight in the F_3 generation provides a further point of contrast, both to males and to F_1 females; larger adults occurred where May weather is sunnier.

All seven environmental variables picked out by regressions with a measure of adult size are significantly inter-correlated (Table 4). This suggests that irrespective of whether the regression analysis picks out a tempera-

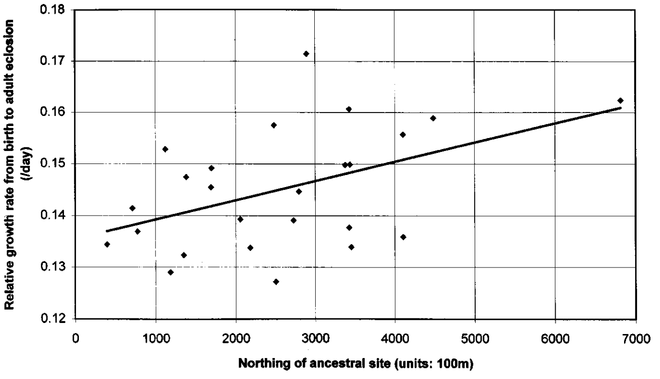

Fig. 5 Growth is faster in the north, as shown by the regression of relative growth rate from birth to adult eclosion (mean + residual after regression against sequence) against northing of ancestral site. See Table 1 for regression statistics

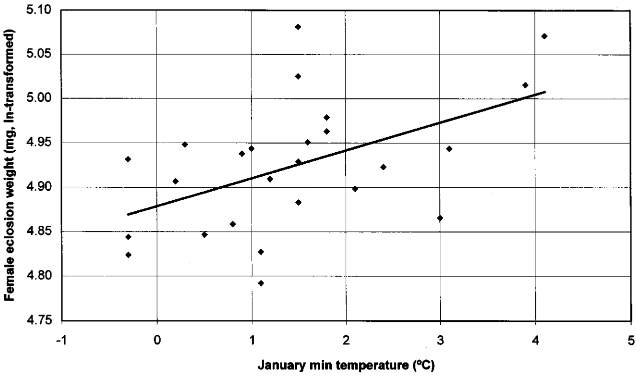

Fig. 6 Female eclosion weight is greater where winters are mild, as shown by the regression of female eclosion weight (mean + residual after regression against sequence) against January minimum temperature. See Table 2 for regression statistics

Table 1 Summary statistics for the partial regressions of each of five female life history variables (all measured on the F_1 generation in 1987) against northing (units: 100 m) of grasshopper collection site, controlling for the effects of rearing sequence. Data on the proportion of individuals inserting the IIa instar were arcsine transformed prior to analysis. Standardised regression coefficients (β), are presented to allow comparisons between tests on different variables. Adjusted R^2 values are used to give an unbiased estimate of goodness of fit

Female life history variable	β	Adjusted R^2	*t*	*df*	*P*
Weight after second moult (mg)	0.53	0.20	2.59	17	0.019
Proportion inserting IIa instar	−0.56	0.26	−3.22	23	0.004
ln[nymphal development period (days)]	−0.42	0.58	−3.23	23	0.004
Relative growth rate to adult eclosion (day^{-1})	0.32	0.60	2.54	23	0.018
Final weight (mg)	−0.47	0.17	−2.50	22	0.020

Table 2 Regression statistics for the seven significant partial regression relationships found with meteorological variables for female life histories, controlling for the effects of rearing sequence (see legend to Table 1 for more details)

Female life history variable	Meteorological variable	β	Adjusted R^2	*t*	*df*	*P*
Egg viability (1987–1988) (%)	June maximum temperature (°C)	0.67	0.39	4.08	21	0.0005
Female hatchling weight (1987) (mg)	Annual maximum temperature (°C)	−0.49	0.17	−2.60	22	0.0162
Hatchling weight (1989) (mg)	August maximum temperature (°C)	−0.61	0.31	−3.58	22	0.0017
ln(female eclosion weight 1987) (mg))	January minimum temperature (°C)	0.52	0.22	2.81	22	0.0103
Female eclosion weight (1989) (mg)	May sunshine (h day^{-1})	0.75	0.52	4.61	16	0.0003
Female mature weight (1987) (mg)	December maximum temperature (°C)	0.54	0.19	2.68	21	0.0141
Female femur length (1987) (mm)	December maximum temperature (°C)	0.59	0.24	3.05	21	0.0061

Table 3 Regression statistics for the five significant partial regression relationships found with meteorological variables for male life histories, controlling for the effects of rearing sequence (see legend to Table 1 for more details)

Male life history variable	Meteorological variable	β	Adjusted R^2	*t*	*df*	*P*
Hatchling weight (1987) (mg)	Annual maximum temperature (°C)	−0.48	0.16	−2.57	22	0.018
ln(nymphal development period 1987) (days)	February minimum temperature (°C)	0.38	0.41	2.39	22	0.026
ln(eclosion weight 1987) (mg)	June sunshine (h day^{-1})	0.54	0.27	2.85	18	0.011
Mature weight (1987) (mg)	September sunshine (h day^{-1})	0.53	0.19	2.40	15	0.030
Final weight (1987) (mg)	April minimum temperature (°C)	0.46	0.19	2.42	21	0.025

ture variable, a sunshine variable or national grid northing in particular cases, there is a single underlying trend towards larger adults in more southern, warmer and sunnier localities.

Discussion

Intraspecific variation

There was remarkable variation in life history between the populations (Figs. 2, 3, 4, 5 and 6). After rearing under standardised laboratory conditions, it may be assumed that maternal effects, as a source of variation between populations, had been reduced (Mousseau and Dingle 1991), and that the phenotypic life history differences observed represent genetic differences between populations.

Intra-specific genetic differences in insects are rarely studied, except in relation to overall morphology and colour variation, with an emphasis on discrete morphs or subspecies. These results add to a growing body of evidence for intraspecific genetic diversity of insect life histories (Clausen and Hiesey 1958; Hewitt and John 1970; Hewitt and Ruscoe 1971; Atkinson and Begon 1987b; Grant et al. 1993; Dingle and Mousseau 1994; Partridge and French 1996; Mousseau 1997; Partridge and Coyne 1997; Sibly et al. 1997). As noted above, a much greater body of work exists on ecotypic differentiation in plants (e.g. Heslop-Harrison 1964; Briggs and Walters 1997).

Temporal resolution of climate regressions

The temporal resolution of climate regressions must be treated with some caution. Of the 17 significant partial regressions, 10 are with meteorological data for particular months. However, to conclude that the weather in that month exerts a selective force on the trait in question could be erroneous. Climatic variables are often strongly inter-correlated. Very high correlations are typical between adjacent months, and data for adjacent months are not always fully independent (e.g. the same weather system may affect consecutive months).

The regressions above illustrate this problem. It is to be expected that a trait of a particular life stage would be affected most significantly by climate during that stage, e.g. nymphal traits would be affected by climate during May and June. However, of the ten partial regressions with the climate of particular months, only four correspond with the season of the life stage concerned. Adults may be found from June until September or October, and yet adult size measures are correlated with climate in January, April, May, June, September and December. All these climate variables, as well as northing, are significantly correlated with each other (Table 4). Thus, the temporal resolution of these regressions is unreliable; they may be used to identify broad seasons where regressions are strongest, but cannot be used to identify the month with the strongest effect.

Climatic effects on life history variables

Hatchling size

As hypothesised, the heaviest hatchlings do indeed come from the coldest sites but the analysis did not resolve which season had the strongest effect. The regressions of hatchling weights on climate variables are different from the regressions for the various measures of adult size. This suggests that inter-population variation in hatch-

Table 4 Correlation coefficients, n, and significance level (P) of correlations between all environmental variables which were selected as significant partial regression predictors of a measure of adult size

	January minimum temperature (°C)	December maximum temperature (°C)	June sunshine (h day^{-1})	September sunshine (h day^{-1})	April minimum temperature (°C)	May sunshine (h day^{-1})
Northing (100 m)	−0.51 26 $P = 0.007$	−0.70 26 $P < 0.0005$	−0.64 23 $P = 0.001$	−0.75 23 $P < 0.0005$	−0.44 26 $P = 0.023$	−0.67 23 $P = 0.001$
January minimum temperature		0.89 26 $P < 0.0005$	0.55 22 $P = 0.008$	0.52 22 $P = 0.013$	0.94 26 $P < 0.0005$	0.69 22 $P < 0.0005$
December maximum temperature			0.60 22 $P = 0.003$	0.58 22 $P = 0.005$	0.76 26 $P < 0.0005$	0.72 22 $P < 0.0005$
June sunshine				0.90 23 $P < 0.0005$	0.57 22 $P = 0.005$	0.97 23 $P < 0.0005$
Sept. sunshine					0.55 22 $P = 0.008$	0.87 23 $P < 0.0005$
April minimum temp.						0.70 22 $P < 0.0005$

ling weights occurs as a direct result of selection for the environments experienced by eggs and hatchlings, rather than indirectly by selection on correlated maternal traits.

That the weight of hatchlings is related most strongly to maximum rather than minimum temperatures is to be expected. Maximum temperatures are effectively daytime temperatures: cool daytime temperatures inhibit feeding, growth, development and the ability to avoid predation. By contrast, night temperatures may only affect fitness if they exceed cold tolerance limits. Thus, it is the pervasive effects of daytime temperatures rather than extreme nocturnal temperatures which may be expected to have greater fitness consequences.

Egg viability

Egg survival (viability) is probably very rarely subject to optimising, rather than maximising, selection. From this perspective, the variation in egg viability among these populations may be interpreted as reflecting differential favourability of the egg treatment regime for different populations.

Smaller eggs may be predicted to suffer higher mortality as a result of their reduced provisioning, perhaps with a compensatingly larger number of eggs being produced. Under this scenario, viability would be poorest in populations with smaller hatchlings, but no such correlation is apparent in the data from 1987–1988. In fact, egg viability was greater in southern populations where eggs tend to be smaller. It seems most probable that more southerly populations are better adapted to the temperatures experienced by the eggs in the laboratory. The over-wintering temperature of 4°C and the fluctuating temperature regime of 20/30°C after over-wintering are probably rather high compared to soil temperatures in the field.

Juvenile growth and development rates, and adult size

Three hypotheses have been proposed for the relationships of juvenile growth rate, nymphal development rate and adult size to climate. All assume that juvenile growth rate is unlikely to vary significantly between populations, under a single environment. Larger adults will be favoured by (1) longer seasons and (2) reduced juvenile mortality rate (both scenarios which permit delayed maturation), or by (3) conditions which permit faster growth.

Season length is difficult to define, and harder still to relate to the available climate data. Monk (1985) and Hartley and Warne (1973) estimate the season lengths of orthopterans as accumulated day-degrees above a threshold temperature. However, in addition to warm daytime temperatures, grasshoppers require sunshine and frost-free nights. The threshold requirements of warmth and sunshine, and the tolerance of cold are not precisely known, nor are the effects of temperature, sunshine and precipitation on juvenile mortality rates. Moreover, these physiological variables seem likely to vary over the species' range.

In practice, these three hypotheses are probably indistinguishable using the current data set, since available indices of season length, indices of reduced juvenile mortality and indices of favourable growth conditions are very likely to be inter-correlated. For example, sites with sunnier summers are probably also sunnier for a longer period and climatically induced mortality is probably less.

Grasshoppers from more northern populations grew faster to adulthood (Fig. 5), against expectations that growth rates would be relatively invariant between populations; populations were predicted to share a single thermal reaction norm for growth rate. We suggest two interpretations for this observation. First, the original hypothesis may be wrong, and different populations may have different thermal reaction norms for growth. Second, the original hypothesis may be valid, but northern grasshoppers may exhibit greater basking efficiency, attaining higher body temperatures and permitting faster growth (M.G. Telfer and M. Hassall, unpublished data).

Development is faster among northern grasshopper populations. The additional IIa instar (Hassall and Grayson 1987) is inserted less frequently by more northerly populations (Fig. 3) and the total duration of nymphal development is shorter (Fig. 4). These are predicted responses to the shorter season length, potentially higher juvenile mortality rate and unfavourable growth conditions of more northerly latitudes.

The seven different measures of adult size correlate with seven different environmental variables (Tables 1, 2 and 3). These regressions defy precise interpretation. For example, a genuine causal relationship between mild winters and large adult size (Fig. 6) seems very unlikely, especially since diapausing eggs are less sensitive to environmental conditions than any other life stage (Cherrill 1987).

Of the remaining climate variables, June sunshine would provide favourable growth conditions, and thus larger adults. Furthermore, September sunshine, April minimum temperature and May sunshine may all be indicators of a longer season; warmer and sunnier during the spring and autumn months. However, the range of environmental variables related to adult size, and their inter-correlations (Table 4), makes discrimination between the three hypotheses impractical.

In summary, northern grasshoppers hatch larger and grow faster than their southern conspecifics. However, the shorter nymphal development periods result in relatively smaller adults than in more southern, warmer, sunnier localities. This trend to smaller size at higher latitudes has been demonstrated before in Orthoptera (Mousseau 1997). The usual trend among ectotherms is for increased size at higher latitudes (Partridge and French 1996). Latitudinal trends generally mirror temperature-related trends, which have been the subject of much more intensive investigation (Atkinson 1994, 1995, 1996).

The importance of climate

Within the British Isles, *C. brunneus* is distributed across a landscape where climate varies greatly. It is known that life history may vary substantially due to local variations in sward structure and thus microclimate (Grayson 1984). It is now clear that macro-climatic variation also exerts a significant influence.

Climate affects many components of biological communities. Its effects on grasshopper life histories may be indirect, through effects on predators, pathogens (Carruthers et al. 1992), parasites, competitors or food. Such indirect effects would probably be non-linear and highly variable in space and time, tending to obscure regressions with climate data. It seems more likely that these regressions do indicate direct climatic effects on life history evolution.

Conclusions

Across the British Isles, grasshopper populations exist with different life histories. The life history differences are, at least partially, under genetic control. Climate obviously represents a major axis of environmental variation between populations, but it can now be appreciated that climate exerts powerful selective forces on populations across the country. This is in accordance with the views of Uvarov (1931) and Lawton (1995) on the importance of climate to insects.

Few previous studies of animals have taken such a large-scale approach to the study of intraspecific life history variation in so many discrete populations. The resulting discovery of a fine-grained patchwork of grasshopper ecotypes evolving under regional conditions offers a valuable insight into biodiversity and is likely to be applicable to a wide range of organisms. It suggests relatively rapid adaptation to local conditions by highly dispersive and rapidly fragmenting post-glacial founder populations. Responses to anthropogenic and climate change in the future may depend on the ability of each genotype to undergo a further period of rapid evolution.

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