Oecologia (2006) 149:245–255
DOI 10.1007/s00442-006-0434-z

COMMUNITY ECOLOGY

Body size patterns in *Drosophila* inhabiting a mesocosm: interactive effects of spatial variation in temperature and abundance

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Received: 9 January 2006 / Accepted: 29 March 2006 / Published online: 14 June 2006

Abstract Body size is a major component of fitness. However, the relative contributions of different factors to optimal size, and the determinants of spatial and temporal variation in size, have not been fully established empirically. Here, we use a mesocosm of a Drosophilidae assemblage inhabiting decaying nectarines to investigate the influence of spatial variation in temperature on adult body size in *Drosophila simulans* Sturtevant. Two treatments were established; one in the sun where developing larvae were exposed to high temperatures and the other in the shade where temperature conditions were milder. The simple developmental effects of temperature differences (i.e. larger flies are likely to emerge from cooler environments), or the simple effects of stressful temperatures (i.e. high temperatures yield wing abnormalities and smaller flies), were overridden by interactive effects between temperature and larval density. Emergences were lower in the sun than shade, probably as a result of temperature-induced mortality. However, flies attained the same final sizes in the shade and sun. In addition, abnormally winged flies were clustered in the shaded treatments. In the shade treatments, where emergences were higher than in the sun, stressful conditions as a result of high larval density likely resulted in wing abnormalities and small size. Consequently, there was little spatial variation in size across the mesocosm, but substantial spatial variation in abundance. Under natural conditions both mortality and non-lethal effects of temperature and/or crowding are likely to play a role in the evolution of body size.

Keywords Abundance · Crowding · Spatial autocorrelation · Stress

Communicated by Thomas Hoffmeister

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1 Introduction

It is well established theoretically that resource availability and quality, production rate, competition, the likelihood of mortality, and the length of the growing season contribute to optimal (≈fitness maximizing) size at first reproduction (reviews in Roff 2002; Kozłowski et al. 2004). However, the relative contributions of these factors to optimal size have not been fully established empirically (Blanckenhorn 2000; Angilletta et al. 2004). In particular, the determinants of spatial and temporal variation in size are poorly investigated for most insects (Kari and Huey 2000; Blanckenhorn and Demont 2004). Nonetheless, considerable empirical work has been done on the proximate and ultimate determinants of body size, both in the laboratory and in the field, using *Drosophila* species as model organisms.

In the laboratory, the effects on *Drosophila* body size of various factors, such as temperature (Partridge

et al. 1994; Pétavy et al. 2001), larval crowding (Delcour and Lints 1966; Santos et al. 1994), food concentration (De Moed et al. 1997), ethanol concentration (Hageman et al. 1990), and desiccation (Gibbs and Matzkin 2001), have been widely investigated. This work has regularly included examinations of the proximate determinants of size variation (Partridge et al. 1994; French et al. 1998; Azevedo et al. 2002), and the effects of this variation on, for example, fecundity, longevity and developmental time (Zwaan et al. 1992; James and Partridge 1995; McCabe and Partridge 1997; Bangham et al. 2002).

Field investigations have revealed that mechanisms similar to those identified in the laboratory underpin size variation. These include spatial and seasonal variation in temperature, variation in resource and water availability, and abundance effects via crowding and resource appropriation or alteration (e.g. Atkinson 1979; Barker 1983; Coyne and Beecham 1987; Thomas 1993; Worthen et al. 1993; James and Partridge 1995, 1998; Borash et al. 1998; Karan and Parkash 1998; Jenkins and Hoffmann 2000; Kari and Huey 2000).

Despite substantial recent progress in reconciling laboratory and field findings, several difficulties stand in the way of integrating these findings (e.g. Weeks et al. 2002). For example, it is clear that laboratory and field flies differ in several ways, including size (David et al. 1997; Jenkins and Hoffmann 2000), longevity (Boulétreau 1978), and the responsiveness of size to directional selection (Gibbs and Matzkin 2001). Thus, whilst laboratory studies generally, and rightly, examine variation in the factor of interest whilst holding all others constant, they offer model organisms an environment very different to the one they are likely to experience naturally. This in turn might make the findings of laboratory studies incompatible with the situation in the field. For example, *D. melanogaster* evolves increased water content under desiccation in the laboratory, but this trait is not typical of xeric species, probably because of manoeuvrability problems associated with larger size (Gibbs et al. 1997; Gibbs and Matzkin 2001). By contrast, field studies, and especially those undertaken over large spatial and temporal scales, have to contend with multiple interacting factors, such as water and resource availability, temperature, day length, parasitism, and abundance (Borash et al. 1998; Houle and Rowe 2003). For example, whilst rapid development is likely to increase fitness by decreasing the time that eggs and larvae are exposed to parasitoids and declining resource quality (James and Partridge 1995), it might also lead to a decrease in body size and therefore greater susceptibility to starvation (Chippindale et al. 1996).

Therefore, even when field patterns seem to reflect those found in the laboratory, and correlative studies reveal potentially similar mechanisms, ascertaining the causal factor(s) underlying size variation remains problematic. In consequence, calls have recently been made for investigations of the interactions between the various mechanisms that are likely to affect life history variables, such as body size, under controlled field conditions (Jenkins and Hoffmann 2000; Pétavy et al. 2001; Hoffmann et al. 2003a). One effective way of combining the control of laboratory studies with the more realistic conditions of the field is by using a mesocosm approach (Srivastava et al. 2004). Here, factors of interest can be intentionally manipulated in a controlled fashion whilst others remain a function of the ''natural'' environment.

In this study we use a mesocosm experiment, consisting of a regular lattice of nectarine fruit exposed to sun or shade (Warren et al. 2003), to investigate the influence of spatial variation in temperature on adult body size in *Drosophila simulans* Sturtevant. Spatial variation in resource quantity and other abiotic variables is effectively constant because they show very similar natural temporal variability across the spatial treatment. Moreover, rather than fixing abundance per resource unit, this is allowed to vary, but is measured, so enabling us to investigate interactions between abundance and temperature on final body size. Based on what is known of the effects of both temperature and abundance on adult body size in *Drosophila* we made the following predictions. In the absence of interactive effects, larger adult flies are expected to emerge from shaded fruit by comparison with those emerging from unshaded fruit, owing to the effects of temperature on size (Atkinson 1994; David et al. 1997). Flies in unshaded fruit might also be characterized by higher levels of developmental abnormality and smaller body size if unshaded fruit represent a stressful environment because of high temperature (Hoffmann et al. 2003b). The typical response in *Drosophila* to high temperature is the expression of heat shock proteins, or their diversion from normal developmental regulation (Rutherford and Lindquist 1998; Hoffmann et al. 2003b), for protein chaperone purposes. Both of these processes interfere with normal growth and development, and would result in smaller body size and a high incidence of developmental abnormalities (see Roberts and Feder 1999). Thus, the ''main effects'' expectation is for smaller adult size and greater developmental abnormality in the sun treatments than in the shade. However, interactive effects may also influence adult body size. Temperature-induced mortality in unshaded fruit, or oviposition avoidance in hot

fruit, lowers larval density relative to shaded fruit and therefore the effects of crowding are relaxed in unshaded fruit (leading to greater resource availability and quality). Larvae that are able to either find thermal refuges or that have a higher stress resistance in unshaded fruit might then be capable of reaching a large body size because of improved resource availability or quality compared to shaded fruit. This is the ''interactive effects'' expectation. Distinguishing the main effects from the interactive effects expectations is relatively straightforward in terms of size patterns. In the former case spatial variation should be strong and show larger sizes in shaded treatments. By contrast, in the latter case, either weak or little spatial pattern in body size associated with shade and sun treatments is to be expected, or the spatial pattern might be the converse of that found for the main effects expectation. The main reason for the complex pattern in the latter case is that the effects of competition and temperature are not likely to be symmetric.

2 Materials and methods

2.1 Experimental design and sampling procedure

A Drosophilidae assemblage was allowed to naturally colonize a mesocosm (the ''study arena'') comprising decaying nectarines (*Prunus persicae* Miller variety *nectarina*: Rosaceae) (Warren et al. 2003). The study arena comprised a grid of 12×18 fruit, spaced 20 cm apart, and placed on a wire table at the University of Pretoria's Experimental Farm, South Africa (25°45′S 28°15′E). The grid was divided into six plots of 36 nectarines, each nectarine on a coarse plastic mesh in the centre of a round plastic container (~15 cm diameter and 8 cm deep) containing washed, moist sand. Three of the plots were artificially shaded (15 cm above the fruit) with 80% shade netting in a checkerboard design to impose variation in the microclimate to which the fruit and therefore the fly larvae were exposed (Warren et al. 2003). The table was placed inside a wire-covered cage (mesh size ~ 0.7 cm) to exclude birds, fruit-piercing moths and large wasps, and ants were excluded by the application of grease to the table legs. Nectarines were washed and weighed to estimate the resource quantity per fruit, before being placed in the field. Initial fruit mass did not vary between treatments (sun, mean±SE=58.59±1.09 g; shade, mean±SE=59.08±1.07 g; t=0.35, df=214, P=0.72). Five nectarines were randomly selected for insecticide-residue tests and were found to have no detectable levels of residues of the organophosphates, organochlorides and pyrethroids used in the local soft fruit industry (South African Bureau of Standards). Three small puncture holes were haphazardly made in the skin of the fruit before placement in the field because *Drosophila* species do not lay eggs on unbroken fruit surfaces (Atkinson 1983; Feder and Krebs 1998).

Six copper-constantan thermocouples were placed 1 cm deep under the skin of six nectarines to measure fruit temperature. Three nectarines in one of the shaded plots and three nectarines in one of the exposed (sun) plots were selected (from the edge of a treatment plot to the interior) to represent the range of temperatures experienced by the flies occupying the fruit. Temperature measurements were taken every 10 min for the duration of the experiment. The mean vapour pressure deficit for each day (mean VPD) was calculated for the study arena using a non-aspirated psychrometer (for rationale see Unwin and Corbet 1991; Al-Saffar et al. 1995). Although rainfall (millimetres per day) was recorded using a tipping-bucket rain gauge at the site, rainfall was strongly correlated with mean VPD (r_s=–0.77, $P < 0.05$), and was excluded in all analyses. An R. M. Young wind sensor was used to measure wind speed. All data were recorded (at 10-min intervals) by a Campbell Scientific CR10 data logger. The experiment ran for 25 days in November 1998.

Because temperature changes during larval development may influence the adults that finally emerge from the fruit, nectarines remained in the field for the duration of the experiment. At the pupal stage, the insect has already consumed the food required to become an adult. Thus, removal of the pupae from the field will not influence the linear dimensions or species composition of the emerging adults. Pupation was expected to take between 4.5 and 8.5 days for flies at 25°C and 80% relative humidity (Sevenster and Van Alphen 1993). Every second day for 25 days, starting from the fifth day after laying out the experiment, the sand under the nectarines containing the fly pupae was removed and placed in 350-ml jars. This was repeated 11 times. Fresh, moist sand was then placed into the containers under the fruit. The sand was kept moist by spraying a standard volume of water onto it each day. The jars were then taken to the laboratory and the emerging flies were recorded, identified and measured. Examination of the sand suggested minimal, if any, pupal mortality. At least six Drosophilidae species were found (Warren et al. 2003), but *D. simulans* Sturtevant dominated the samples (96% of all flies).

Important considerations in a study such as this are the time of termination of sampling, and the likelihood of uneven truncation of the data between the two treatments (i.e. differential emergence of flies from sun

and shaded treatments beyond the days for which sampling was undertaken). By the last sampling day of this experiment the fruit were blackened, completely dried and shrivelled and we expected relatively few additional flies to emerge (a later experiment revealed this to be the case; data not shown). More importantly, for a truncated data series to have substantially influenced the present study, the proportional decrease in the number of flies emerging from the unshaded treatments would have had to be lower than that for shaded fruit (i.e. a longer tail in the emergence from the unshaded fruit). In an additional experiment (data not shown here) the proportional decrease in fly abundances in the unshaded treatments was generally higher than in the shaded treatments. Therefore, artificial data truncation is unlikely to have influenced the current study to any great extent.

2.2 Data

Thorax length was used as a measure of size (Cowley and Atchley 1990). It was determined with an ocular micrometer on a binocular microscope, to the nearest 0.01 mm, from the anterior margin of the thorax to the posterior tip of the scutellum as viewed from the side. All flies were sexed. Abnormal wing development was taken as an indication of stressful developmental conditions. Flies were scored as either: no wing abnormality, normal wings(W0); slight curling of one or both wings (W1); severe curling of one or both wings (W2) [similar abnormalities were reported by Roberts and Feder (1999)].

Excluding flies with thoracic and abdominal damage, 6,849 of a total of 7,228 *D. simulans* individuals were measured. No flies and two flies were recorded for sampling days 5 and 7, respectively, and these days were therefore excluded from the analyses.

2.3 Analyses

To demonstrate that temperatures in the sun and shade treatments differed, means, minima, maxima and ranges were calculated for each thermocouple across the experimental time period. Treatment-associated differences in these temperature parameters, as well as differences in the time (number of hours) that fruit in the sun and shade were exposed to temperatures above 32°C (Hsp induction takes place above 32°C, Hoffmann et al. 2003b), and above 37°C (lethal for drosophilids, Feder and Krebs 1998) were assessed using Mann–Whitney *U*-tests (Quinn and Keough 2002).

To distinguish the interactive and main effects expectations, the spatial pattern of body size variation [sum of all thorax lengths, which is also a surrogate for, and perhaps a better measurement of, abundance—see Krijger et al. (2001), and mean thorax length per fruit] across the study arena was investigated using spatial autocorrelation (SAAP version 4.3, Wartenberg 1989). Spatial autocorrelation (or spatial dependence) refers to the tendency of spatially distributed variables to be more similar the closer they are to one another, and more dissimilar as the distance between them increases. We expected that thorax length variables would be positively autocorrelated for the distance corresponding to the size of a treatment plot (0.00–1.08 m) and for the distances corresponding to adjacent plots of the same treatment (2.3–2.7 m), while thorax lengths would be negatively autocorrelated across the distance between adjacent plots (1.08–2.3 m) if the sun/shade treatments were driving spatial pattern (see also Sokal and Wartenberg 1983). Omnidirectional correlograms of the autocorrelation coefficient (Moran's *I*) as a function of distance were drawn. Fifteen distance classes were chosen with equal distance intervals (each 0.27 m). Because distance classes with fewer than 1% of the total number of sample pairs in a class should not be interpreted, correlograms were drawn for distance classes 1–10 only (Legendre and Fortin 1989). Thorax length variables were transformed (ln) to stabilize the variance prior to analysis (Legendre and Legendre 1998). The correlograms were tested for global significance, i.e. the correlogram contains at least one value that is significant at the Bonferroni corrected level (Legendre and Legendre 1998).

Spatial patterns in wing abnormalities of individuals were investigated using spatial analysis by distance indices (SADIE) (Perry et al. 1996). This method is advocated for count data with zeros (Perry 1995) and for this reason is particularly suited to the analysis of the wing abnormality counts. An index of aggregation (I_a) was calculated for each wing abnormality category using the SADIE randomization procedure (Perry 1995). Values of $I_a > 1.0$ indicate spatial aggregation, those approximating 1.0 indicate randomness, and those < 1.0 indicate regularity (Perry 1995).

Indices of aggregation provide limited information on spatial pattern. SADIE, however, is also able to explicitly incorporate spatial information associated with samples (localities) into the quantification of spatial pattern. SADIE was used to examine spatial aggregation in wing abnormality categories at individual localities (fruit) (Perry et al. 1996, 1999). SADIE is able to identify patches ($v_i > 1.5$; areas where clusters of high counts occur) and gaps ($v_j < -1.5$; areas where clusters of low counts occur) (Perry et al. 1999). The v_i

and v_j values for each fruit for wing abnormality categories were plotted to visually inspect clustering across the study arena (Perry et al. 1999), and the average patch $(\overline{v}_i)$ and gap $(\overline{v}_j)$ distances were calculated to formally test for clustering in wing abnormality counts. This analysis was only performed for W0 and W2. Spatial patterns in W1 were not investigated because these flies were not abundant (n=80) and were found on a few fruit only. Under these conditions type II error rates may be high (Korie et al. 2000).

Prior to regression analysis, the potential redundancy of the independent variables [sampling day, sex, wing abnormality (normal or abnormal, i.e. after merging categories W1 and W2), mean VPD per day, treatment (sun or shade), fruit mass and total abundance] were examined for the full dataset (sampling days 9–25) using the tolerance approach advocated by Quinn and Keough (2002). Tolerance may be calculated as $1–R^2$ for the respective variable with all the other variables in the equation. Tolerance values close to zero reflect that the variable is likely to be redundant, leading to collinearity between the variables. Including such a variable in the model falsely inflates the significance of the model or the amount of variation ''explained'' by the model. In general, tolerances for the above variables were high (>0.83) indicating that collinearity was unlikely to compromise the outcomes of the generalized linear models (GLZ) (see below).

GLZ with normal error structure and identity link function were used to evaluate the effects of sampling day (continuous variable), VPD, treatment, sex, abundance, fruit mass, wing abnormality, and their interactions, on individual thorax length for the full dataset (days 9–25) (STATISTICA version 5.5; McCullagh and Nelder 1989). The best subsets likelihood ratio approach was used to determine the best-fit model with fewest terms (McCullagh and Nelder 1989; Collet 1991; Dobson 2002). Goodness of fit was measured using the deviance statistic and the percentage deviance explained (similar to R^2) for the best fitting model was calculated. The change in deviance for single variables was used to estimate the contribution of individual variables to the deviance explained by the final model (Collet 1991).

3 Results

Over the study period, wind speed was low (1.0±0.8 m s^{-1}, mean±SE), and a total of 102 mm rainfall fell on 10 days. The ambient environment was relatively stable from sampling days 17 to 25. Mean VPD prior to sampling day 13 was often higher than for the remaining sampling days. The VPD dropped drastically at sampling day 13 and then restabilized, fluctuating around 0.7 kPa until the completion of the experiment. A clear successional pattern in fruit decomposition was observed from initial placement of the fruit in the field to the final (25th) sampling day when fruit were blackened, shrivelled and completely dried out. Temperatures differed significantly between sun and shade treatments. The temperature range in the sun treatment was 10°C larger than in the shade (Table 1). Daily temperature regimes of the fruit in the sun and shade treatments were similar, although the maximum temperatures in the sun were significantly higher than those measured in the shade (Table 1). More importantly, the time that fruit were exposed to temperatures above 32°C and above 37°C was significantly longer for the sun (median=86 and 48 h, respectively) than for the shade (median=23 and 3 h, respectively) treatments (Table 1).

The abundance of emerging flies was low for sampling days 9–17 in the sun (Fig. 1). It increased over sampling days 19–25 with the highest abundance recorded for sampling day 23 in both the sun and shade.

3.1 Regression analyses

The sum of thorax lengths of all individuals per fruit was spatially structured, with higher values for the shade treatments. By contrast, mean thorax length per fruit did not display significant spatial structure (Fig. 2). Thus, although the spatial structure in the sum of the thorax lengths matched the main effects expectation, controlling for abundance removed the spatial structure. Biologically, this means that similar-sized flies were found in the sun and shaded treatments. In consequence, the main effects expectation was rejected.

The SADIE analysis revealed significant aggregation in wing abnormality scores (Table 2). Aggregation was strongest for W0, and patch and gap indices were

Table 1 Results of Mann–Whitney U-tests for differences in temperature (°C) means, minima, maxima and ranges and mean (±SE) time spent at temperatures above 32 and 37°C for fruit in the sun (n=3) and shade (n=3)

	Sun plots	Shade plots	U	Z	$P<$
Mean (±SE)	20.67±0.09	18.82±0.06	0.00	1.96	0.05
Minimum	6.37	8.19	2.00	–1.09	0.28
Maximum	54.11	46.06	0.00	1.96	0.05
Range	47.73	37.87	0.00	1.96	0.05
Mean time >32°C	85.50±12.27	20.28±7.08	0.00	1.96	0.05
Mean time >37°C	49.44±10.71	5.00±3.61	0.00	1.96	0.05

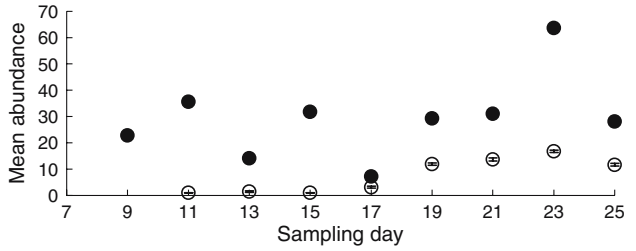

Fig. 1 Mean (±SE) abundance of flies emerging per fruit in the sun (*open circles*) and shade (*closed circles*) on each sampling day. Note that the *error bars* are often hidden by the symbols

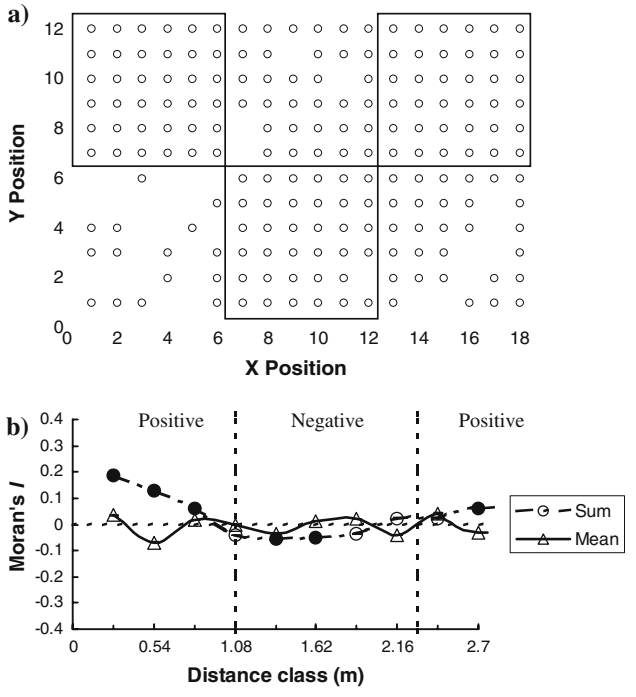

Fig. 2a, b Spatial patterns in thorax length. **a** Map of mean thorax length [interval (0–1) mm] of all flies emerging from each fruit (*outlined blocks* represent plots that were shaded), and **b** spatial dependence (Moran's *I*) in sum and mean thorax length (Bonferroni corrected significance levels α=0.001 and 0.292). *Vertical lines* correspond to sections of correlogram predicted to be positive and negative, respectively, if the spatial dependence in thorax length patterns matches the shaded and unshaded treatments (see text for details). *Filled symbols* indicate significant autocorrelation for that distance class

Table 2 Aggregation indices of gap (area of low counts) and patch (area of high counts) clusters of wing abnormality categories [no abnormality (*W0*), severe wing abnormality (*W2*)] for the pooled data. I_aIndex of patchiness, $\bar{v}_j$ the average value of all gaps, $\bar{v}_i$ the average value of all patches

Data	*n*	I_a	$\bar{v}_j$ (gaps)	$\bar{v}_i$ (patches)
W0	6,340	2.141***	–2.016***	2.122***
W2	427	1.490**	–1.466*	1.55**

*$P<0.05$, **$P<0.01$, ***$P<0.001$

largest and most significant for these flies. In other words, normal winged flies formed patches in the shade (Fig. 3a). Clusters of patches and gaps were also identified for the severest wing abnormalities recorded (W2, Table 2). However, contrary to the main effects expectation, patches of severe wing abnormalities also occurred exclusively in shaded treatments (Fig. 3b). Thus, it appears that for flies surviving to adulthood, developmental conditions were more stressful in the shade than in the sun treatments. This was confirmed by a χ^2-test ($P<0.02$) demonstrating that the observed number of abnormal flies in the shaded treatments was higher than a random assortment predicted from an overall abnormality rate of 7.5%.

The best subset GLZ included sampling day, sex, wing abnormality, mean VPD, and the sex by wing abnormality interaction. It explained 44% of the deviance in thorax length, with the largest contributions being made by sex and wing abnormality (Table 3). Although the thermal environments in the sun and shade treatments were different (Table 1), there was no effect of treatment on thorax length in the full model. Rather, sex and the extent of abnormality had the largest effects on thorax length. The significant interaction term indicated that in females, normal and abnormal winged female flies differed to a much greater extent than in males (Fig. 4). Thus, female flies are larger than male flies, flies with abnormal wings are smaller than those that have normal development, and the effect of the latter was greatest in females. The sun versus shade treatment effect had little significance for thorax size by comparison with exposure to developmentally stressful conditions. Nonetheless, the abundances of flies differed substantially between the treatments (Figs. 1, 5). Approximately 6 times as many flies emerged from the shaded compared to the unshaded fruit (6,264 vs. 1,196).

4 Discussion

Based on what is known of the responses of *Drosophila* species to high temperatures (Hoffmann et al. 2003b), we predicted either that the sun/shade treatment would have a substantial effect on thorax length, resulting in pronounced spatial structure, or that an interaction between abundance and resource quality would result in little, reversed, or no spatial structure in thorax length. No spatial structure in thorax length was found and regression analysis revealed no direct effect of treatment on thorax length. However, patches of high counts of wing abnormalities occurred in the shaded treatments and these were significantly higher than

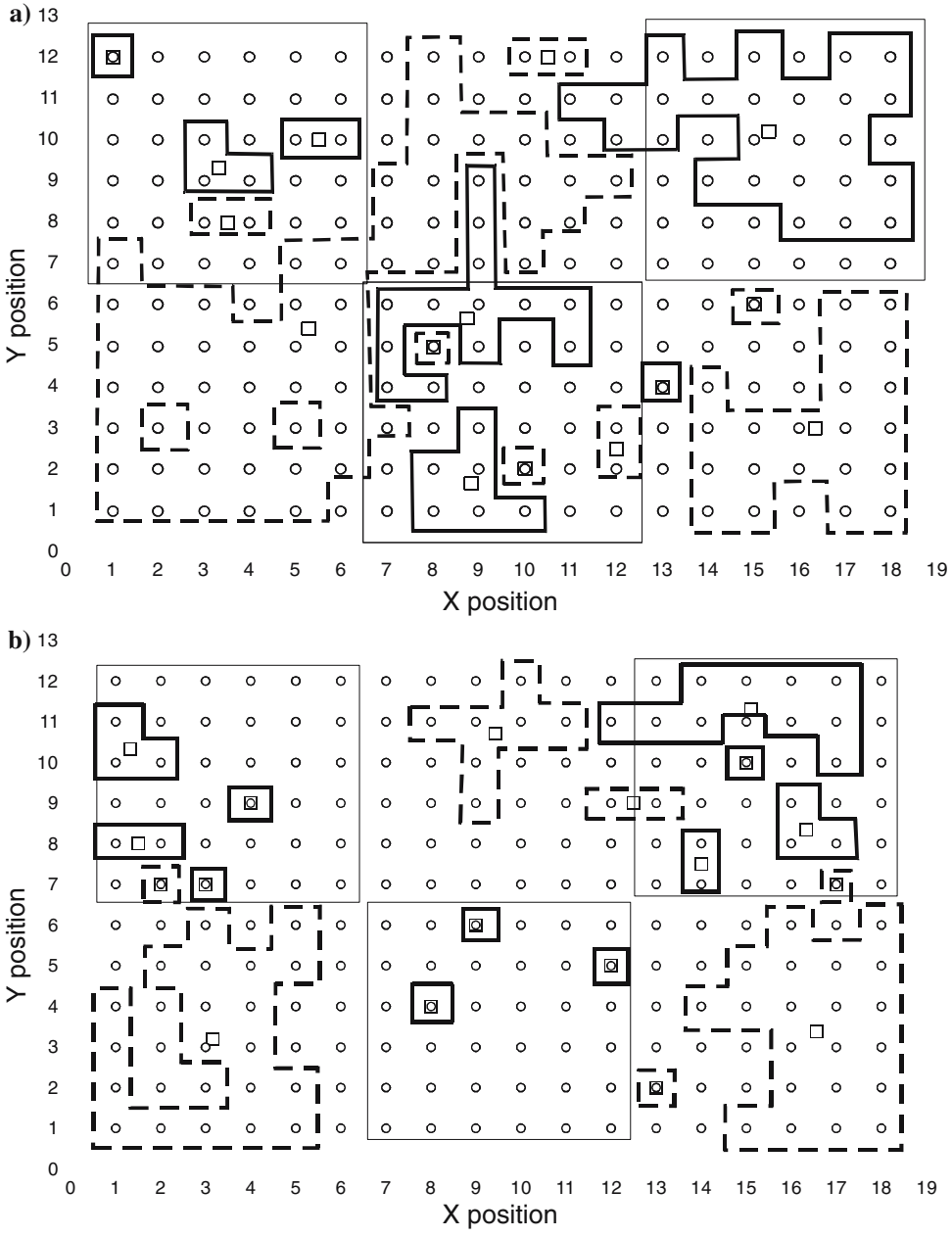

Fig. 3 Spatial positions of abundance patches (*thick solid outline*; *vi*>1.5) and gaps (*dotted outline*; $v_j < -1.5$) for flies with **a** no wing abnormalities, and **b** severe wing abnormalities. *Circles* represent individual fruit; *small squares* represent centroids of patches and gaps; *fine square outline* around groups of circles represents fruit that were shaded by 80% shade netting, with the remainder representing fruit exposed to the sun

expected from the overall number of wing abnormalities. Therefore, in this mesocosm, the developmental effects of temperature differences (Atkinson 1994; David et al. 1997), and/or the effects of stressful temperatures (Hoffmann et al. 2003b), were overridden by more complex interactions.

We also predicted that the relationship between thermal stress and resource quality and availability might form the basis for such an interaction. Thermal stress and the risk of developmental abnormality were likely to have been considerable in the unshaded fruit. However, larvae capable of finding a refuge from high temperatures might also have grown to a large body size, largely because of an absence of larval crowding and competition, or an absence of pre-emptive resource use and subsequent resource pollution (Barker 1983; Scheiring et al. 1984; Hageman et al. 1990; Borash et al. 1998; Sørensen and Loeschcke 2001). On the other hand, large body size may be correlated with greater stress resistance in flies. The results suggest that a combination of low larval densities and stress in the unshaded fruit might have been responsible for the patterns found.

First, abundances of emerging flies were high in the shade and low in the sun, resulting in little difference in mean thorax length between the two treatments. These

Table 3 Best subset generalized linear model for thorax length (mm) and the independent terms sampling day, fruit mass, total abundance, treatment, sex [female (*F*)], wing abnormality [flies with abnormal wings (*ABN*); no abnormality] and mean vapour pressure deficit (*Mean VPD*) for sampling days 9–25. Only variables that were significant are shown. The estimate and the estimated percentage deviance explained (*% Deviation*) by the variables in the model are also provided

Variable	*df*	Log likelihood	χ^2	Estimate	% Deviation	*P*<
Sampling day 9–25 (% deviance explained=43.84, deviance/*df*=0.007, *df*=6841)						
Sampling day	1	7,231.76	468.53	–0.01	3.98	0.001
Sex	1	7,270.37	391.32	0.04^{F}	20.47	0.001
Wing abnormality	1	6,623.61	1,684.82	-0.08^{ABN}	17.62	0.001
Mean VPD	1	7,426.30	79.46	–0.04	1.77	0.001
Sex×wing abnormality	1	7,442.90	46.26	–0.01		0.001

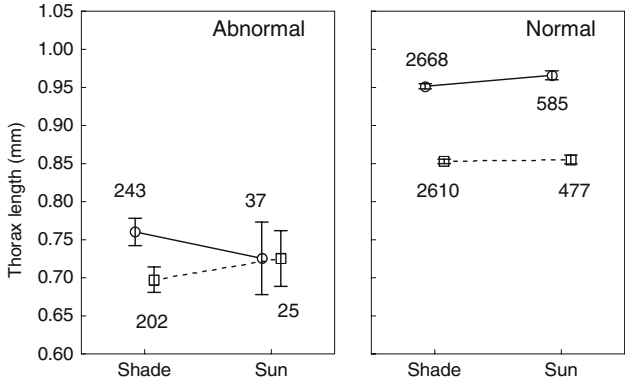

Fig. 4 Interaction plot (mean±SE) showing differences in thorax length [males (*squares*) and females (*circles*)] for abnormally winged and normally winged flies for sampling days 9–25. Sample sizes are indicated

aggregated patterns of emergence, which were driven largely by the treatment, may have been a consequence of egg clustering, female choice, patterns of mortality or some combination thereof (Atkinson and Shorrocks 1984; Heard and Remer 1997; Feder and Krebs 1998; Wertheim et al. 2002). In the field, ovipositing female drosophilids avoid fruit if it is warm at the time of oviposition (Feder et al. 1997). However, they are unable to distinguish between previously heated and unheated fruit under lower temperature conditions (Feder et al. 1997; Feder and Krebs 1998). Therefore, both heat-induced mortality and female choice play a role in determining aggregated emergence patterns (see also Dahlgaard et al. 2001). Thus, the interaction of female choice and heat-induced mortality effect low emergence from unshaded fruit, supporting our interactive effects prediction.

Second, clusters (patches) of wing abnormalities occurred almost exclusively in the shaded treatments (Fig. 3), and the numbers of abnormally winged flies were higher than expected by chance. Therefore, surviving flies in the unshaded fruit were less exposed to stress than those utilizing shaded fruit either because survivors found a thermal refuge or possessed greater temperature stress tolerance. Bubli et al. (1998) and

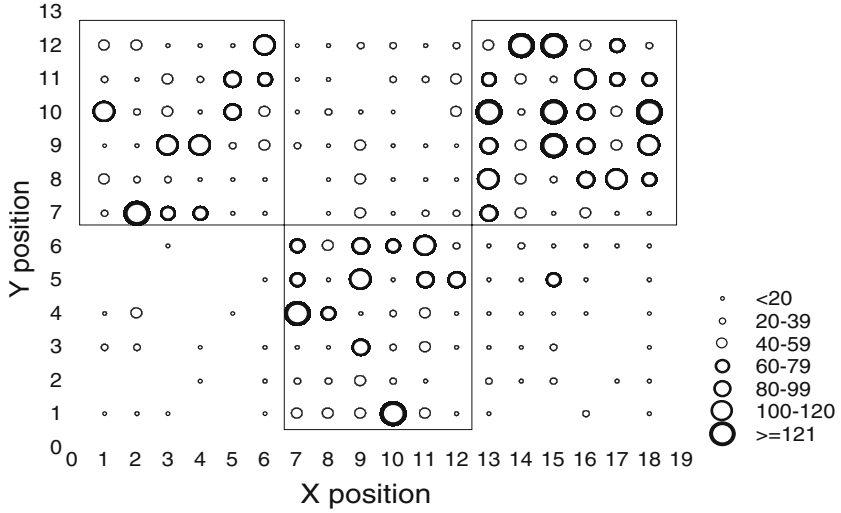

Fig. 5 Spatial pattern in *Drosophila simulans* abundance across the study arena. *Circle size* represents total abundance of flies in individual fruit, *outlined blocks* represent plots that were shaded

Sørensen and Loeschcke (2001) have found expression of heat shock proteins in *Drosophila* experiencing high larval densities. If expression is ongoing as a consequence of high densities, then it might be expected that developmental abnormalities would result because of the diversion of molecular chaperones from their normal regulatory tasks (Rutherford and Lindquist 1998). Moreover, ongoing deterioration of resource conditions as a consequence of high abundances might also mean stressful environments, leading to further developmental abnormality. Thus, wing abnormality is likely to have been a consequence of thermal stress in unshaded fruit, but mostly of crowding in shaded fruit.

In consequence, the interactive effects expectation is more plausible than one of simple, direct effects of temperature. In shaded fruit, competition, resource appropriation and perhaps also resource pollution are likely to have been substantial. Although these conditions did not seem to affect abundances dramatically, they did affect size, both directly (see also Atkinson 1979) and indirectly. The direct effect was relatively small, e.g. by day 25 it was similar to the 7% difference in size due to crowding reported by Sørensen and Loeschcke (2001) for *D. melanogaster*. Indirect effects via growth and development defects were reflected in the presence of abnormalities and the small size of abnormal flies. By contrast, in unshaded fruit emergence was low because of, for example, high mortality, and some influence on wing abnormality was present, but normal winged survivors were able to grow to a size equivalent to that of flies in the shade. In other words, the unshaded fruit were a more favourable density environment (see also Feder et al. 1997) for larvae, whereas the shaded fruit were a more favourable thermal environment. The upshot was little spatial variation in size, but substantial spatial variation in abundance.

Finally, this study has also shown that there is substantial developmental abnormality in flies developing under natural circumstances. In addition, towards the end of the resource lifespan even normal-winged flies can be substantially smaller than those that develop under ideal conditions. Morin et al. (1999) found that under laboratory conditions thorax lengths of *D. simulans* from two populations were 0.96±0.003 and 0.99±0.004 mm in males, and 1.08±0.003 and 1.09±0.004 mm in females, which is substantially larger than the mean values recorded here. Thus, under natural conditions the mortality and non-lethal effects of both temperature and crowding are likely to play a large role in the evolution of body size and need to be given greater empirical attention than has perhaps been the case to date (see Angilletta et al. 2004; Kozłowski et al. 2004).

Acknowledgements Nordi Fruits is thanked for supplying the nectarines. We thank Joe Perry for discussion of SADIE, and Ruan Veldtman for comments on an earlier draft of the manuscript. Two anonymous referees provided helpful and insightful comments on an earlier version of the manuscript. This work was supported by the National Research Foundation (GUN2053618) and by the Mellon Foundation Mentoring Scheme, University of Pretoria.

References

Al-Saffar ZY, Grainger JNR, Aldrich J (1995) Influence of constant and changing temperature and humidity on the development and survival of the eggs and pupae of *Drosophila melanogaster* (Meigen). J Therm Biol 20:389–397

Angilletta MJ, Steury TD, Sears MW (2004) Temperature, growth rate, and body size in ectotherms, fitting pieces of a life-history puzzle. Integr Comp Biol 44:498–509

Atkinson WD (1979) A field investigation of larval competition in domestic *Drosophila*. J Anim Ecol 48:91–102

Atkinson WD (1983) Gregarious oviposition in *Drosophila melanogaster* is explained by surface texture. Aust J Zool 31:925–929

Atkinson D (1994) Temperature and organism size—a biological law for ectotherms? Adv Ecol Res 25:1–58

Atkinson WD, Shorrocks B (1984) Aggregation of larval Diptera over discrete and ephemeral breeding sites: the implications for coexistence. Am Nat 124:336–351

Azevedo RBR, French V, Partridge L (2002) Temperature modulates epidermal cell size in *Drosophila melanogaster*. J Insect Physiol 48:231–237

Bangham J, Chapman T, Partridge L (2002) Effects of body size, accessory gland and testis size on pre- and postcopulatory success in *Drosophila melanogaster*. Anim Behav 64:915–921

Barker JSF (1983) Interspecific competition. In: Ashburner M, Carson HL, Thompson JN (eds) The genetics and biology of *Drosophila*. Academic Press, New York, pp 285–341

Blanckenhorn WU (2000) The evolution of body size: what keeps organisms small. Q Rev Biol 75:385–407

Blanckenhorn WU, Demont M (2004) Bergmann and converse Bergmann latitudinal clines in arthropods: two ends of a continuum? Integr Comp Biol 44:413–424

Borash DJ, Gibbs AG, Joshi A, Mueller LD (1998) A genetic polymorphism maintained by natural selection in a temporally varying environment. Am Nat 151:148–156

Boulétreau J (1978) Ovarian activity and reproductive potential in a natural population of *Drosophila melanogaster*. Oecologia 35:319–342

Bubli OA, Imasheva AG, Loeschcke V (1998) Selection for knockdown resistance to heat in *Drosophila melanogaster* at high and low larval densities. Evolution 52:619–625

Chippindale AK, Chu TJF, Rose MR (1996) Complex trade-offs and the evolution of starvation resistance in *Drosophila melanogaster*. Evolution 50:753–766

Collet D (1991) Modelling binary data. Chapman and Hall, London

Cowley DE, Atchley WR (1990) Development and quantitative genetics of correlation structure among body parts of *Drosophila melanogaster*. Am Nat 135:242–268

Coyne JA, Beecham E (1987) Heritability of two morphological characters within and among natural populations of *Drosophila melanogaster*. Genetics 117:727–737

Dahlgaard J, Hasson E, Loeschcke V (2001) Behavioural differentiation in oviposition activity in *Drosophila buzzatii* from highland and lowland populations in Argentina: plasticity or thermal adaptation? Evolution 55:738–747

David JR, Gibert P, Gravot E, Pétavy G, Morin JP, Karan D, Moreteau B (1997) Phenotypic plasticity and developmental temperature in *Drosophila*: analysis and significance of reaction norms of morphometrical traits. J Therm Biol 22:441–451

Delcour J, Lints FA (1966) Environmental and genetic variations of wing size, cell size and cell division rate, in *Drosophila melanogaster*. Genetica 37:543–556

De Moed GH, De Jong G, Scharloo W (1997) Environmental effects on body size variation in *Drosophila melanogaster* and its cellular basis. Genet Res 70:35–43

Dobson AJ (2002) An introduction to generalized linear models. Chapman and Hall, CRC Texts in Statistical Science, Boca Raton, Fla.

Feder ME, Blair N, Figueras H (1997) Oviposition site selection: unresponsiveness of *Drosophila* to cues of potential thermal stress. Anim Behav 53:585–588

Feder ME, Krebs RA (1998) Natural and genetic engineering of the heat-shock protein Hsp70 in *Drosophila melanogaster*: consequences for thermotolerance. Am Zool 38:503–517

French V, Feast M, Partridge L (1998) Body size and cell size in *Drosophila*: the developmental response to temperature. J Insect Physiol 44:1081–1089

Gibbs AG, Chippindale AK, Rose MR (1997) Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. J Exp Biol 200:1821–1832

Gibbs AG, Matzkin LM (2001) Evolution of water balance in the genus *Drosophila*. J Exp Biol 204:2331–2338

Hageman J, Eisses KT, Jacobs PJM, Scharloo W (1990) Ethanol in *Drosophila* cultures as a selective factor. Evolution 44:447–454

Heard SB, Remer LC (1997) Clutch-size behavior and coexistence in ephemeral-patch competition models. Am Nat 150:744–770

Hoffmann AA, Scott M, Partridge L, Hallas R (2003a) Overwintering in *Drosophila melanogaster*: outdoor field cage experiments on clinal and laboratory selected populations help to elucidate traits under selection. J Evol Biol 16:614–623

Hoffmann AA, Sørensen JG, Loeschcke V (2003b) Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. J Therm Biol 28:175–216

Houle D, Rowe L (2003) Natural selection in a bottle. Am Nat 161:50–67

James AC, Partridge L (1995) Thermal evolution of rate of larval development in *Drosophila melanogaster* in laboratory and field populations. J Evol Biol 8:315–330

James AC, Partridge L (1998) Geographic variation in competitive ability in *Drosophila melanogaster*. Am Nat 151:530–537

Jenkins NL, Hoffmann AA (2000) Variation in morphological traits and trait asymmetry in field *Drosophila serrata* from marginal populations. J Evol Biol 13:113–130

Karan D, Parkash R (1998) Desiccation tolerance and starvation resistance exhibit opposite latitudinal clines in Indian geographical populations of *Drosophila kikkawai*. Ecol Entomol 23:391–396

Kari JS, Huey RB (2000) Size and seasonal temperature in free-ranging *Drosophila subobscura*. J Therm Biol 25:267–272

Korie S, Perry JN, Mugglestone MA, Clark SJ, Thomas CFG, Mohamad Roff MN (2000) Spatiotemporal associations in beetle and virus count data. J Agric Biol Environ Stat 5:214–239

Kozłowski J, Czarnołęski M, Dańko M (2004) Can optimal resource allocation models explain why ectotherms grow larger in cold? Integr Comp Biol 44:480–493

Krijger CL, Peters YC, Sevenster JG (2001) Competitive ability of neotropical *Drosophila* predicted from larval developmental times. Oikos 92: 325–332

Legendre P, Fortin M-J (1989) Spatial patterning and ecological analysis. Vegetatio 80:107–138

Legendre P, Legendre L (1998) Numerical ecology, 2nd edn. Elsevier, Amsterdam

McCabe J, Partridge L (1997) An interaction between environmental temperature and genetic variation for body size for the fitness of adult female *Drosophila melanogaster*. Evolution 51:1164–1174

McCullagh P, Nelder JA (1989) Generalized linear models. 2nd edn. Chapman and Hall, CRC, Fla.

Morin JP, Moreteau B, Pétavy G, David JR (1999) Divergence of reaction norms of size characters between tropical and temperate populations of *Drosophila melanogaster* and *D. simulans*. J Evol Biol 12:329–339

Partridge L, Barrie B, Fowler K, French V (1994) Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. Evolution 48:1269–1276

Perry JN (1995) Spatial analysis by distance indices. J Anim Ecol 64:303–314

Perry JN, Bell ED, Smith RH, Woiwood IP (1996) SADIE: software to measure and model spatial patterns. Asp Appl Biol 46:95–102

Perry JN, Winder L, Holland JM, Alston RD (1999) Red-blue plots for detecting clusters in count data. Ecol Lett 2:106–113

Pétavy G, Moreteau B, Gibert P, Morin J-P, David JR (2001) Phenotypic plasticity of body size in *Drosophila*: effects of a daily periodicity of growth temperature in two sibling species. Physiol Entomol 26:351–361

Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists. Multiple and complex regression. Cambridge University Press, UK, pp 111–154

Roberts SP, Feder ME (1999) Natural hyperthermia and expression of the heat shock protein Hsp70 affect developmental abnormalities in *Drosophila melanogaster*. Oecologia 121:323–329

Roff DA (2002) Life history evolution. Sinauer, Sunderland, Mass.

Rutherford SL, Lindquist S (1998) Hsp90 as a capacitor for morphological evolution. Nature 396:336–342

Santos M, Fowler K, Partridge L (1994) Gene–environment interaction for body size and larval density in *Drosophila melanogaster*: an investigation of effects on development time, thorax length and adult sex ratio. Heredity 72:515–521

Scheiring JF, Davis DG, Ranasinghe A, Teare CA (1984) Effects of larval crowding on life history parameters in *Drosophila melanogaster* Meigen (Diptera: Drosophilidae). Exp Gerontol 77:329–332

Sevenster JG, Van Alphen JM (1993) A life history trade-off in *Drosophila* species and community structure in variable environments. J Anim Ecol 62:720–736

Sokal RR, Wartenberg DE (1983) A test of spatial autocorrelation analysis using an isolation-by-distance model. Genetics 105:219–237

Srivastava DS, Kolasa J, Bengtsson J, Gonzalez A, Lawler SP, Miller TE, Munguia P, Romanuk T, Schneider DC, Trzcinski MK (2004) Are natural microcosms useful model systems for ecology? Trends Ecol Evol 19:379–384

Sørensen JG, Loeschcke V (2001) Larval crowding in *Drosophila melanogaster* induces Hsp70 expression, and leads to increased adult longevity and adult thermal stress resistance. J Insect Physiol 47:1301–1307

Thomas RH (1993) Ecology of body size in *Drosophila buzzatii*: untangling the effects of temperature and nutrition. Ecol Entomol 18:84–90

Unwin DM, Corbet SA (1991) Insects, plants and microclimate. Richmond, Surrey

Warren M, McGeoch MA, Chown SL (2003) Predicting abundance from occupancy: a test for an aggregated insect assemblage. J Anim Ecol 72:468–477

Wartenberg D (1989) SAAP v 4.3—a spatial autocorrelation analysis program. Robert Wood Johnson Medical School, Piscataway

Weeks AR, McKenchnie SW, Hoffmann AA (2002) Dissecting adaptive clinal variation: markers, inversion and size/stress associations in *Drosophila melanogaster* from a central field population. Ecol Lett 5:756–763

Wertheim B, Marchais J, Vet LEM, Dicke M (2002) Allee effect in larval resource exploitation in *Drosophila*: an interaction among density of adults, larvae, and micro-organisms. Ecol Entomol 27:608–617

Worthen WB, Hipp MN, Twardokus CT, Roller RR (1993) Effects of ant predation and larval density on mycophagous fly communities. Oikos 66:526–532

Zwaan BJ, Bijlma R, Hoekstra RF (1992) On the developmental theory of ageing. II. The effect of developmental temperature on longevity in relation to adult body size in *D. melanogaster*. Heredity 68:123–130