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Ecology of body size in *Drosophila buzzatii*: untangling the effects of temperature and nutrition

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Abstract. 1. A method of separating the effects of two important determinants of body size in natural populations, temperature of larval development and level of larval nutrition, by making measurements of thorax length and wing length of adult flies is investigated.

2. I show that at any given time variation in body size of *Drosophila buzzatii* from two sites in eastern Australia is determined primarily by variation in the quality of nutrition available to larvae.

3. Throughout the year adult flies are consistently at least 25% smaller in volume than predicted for optimal nutrition at their predicted temperature of larval development.

4. Nutritional stress is therefore a year-round problem for these flies.

5. Measurements of adult flies emerging from individual breeding substrates (rotting cactus cladodes) show that there is substantial variation among these substrates in the nutrition available to larvae.

6. This method will allow study of spatial and temporal variation in the temperature of larval substrates and in the nutritional resources available to flies in natural populations.

Key words. *Drosophila buzzatii*, body size, developmental temperature.

Introduction

Body size in *Drosophila* species is known to be intimately related to important fitness components such as fecundity, dispersal ability and mating success (e.g. Roff, 1977, 1981; Prout & Barker, 1989; Partridge, 1988). Ecological factors which strongly affect adult body size include quality and quantity of nutrition available to larvae and the temperature of larval development (Robertson, 1963; Atkinson, 1979). Available nutrition in a breeding site is also related to the level of competition among the organisms inhabiting that substrate. The ability to disentangle the effects of temperature and nutrition on adult body size thus allows the tracking at a fine-scale of spatial and temporal variation in resource availability in natural populations.

Robertson (1987) suggested that the ratio of wing length to thorax length, known to be dependent on temperature during juvenile development (e.g. Pantelouris, 1957; Masry & Robertson, 1979; Starmer & Wolf, 1989), may be independent of the level of larval nutrition in *Drosophila buzzatii*. Here I examine this suggestion experimentally and apply the results to a year-long series of collections from two natural populations in Australia. This method allows partitioning of the contributions of temperature during larval and pupal development and resource availability to adult body size over time and between breeding substrates.

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Materials and Methods

Sites. The *D.buzzatii* populations analysed were from three sites, one coastal south Queensland (Hemmant) and two inland New South Wales (Trinkey and O'Hara), Australia. These sites are described in more detail in Thomas & Barker (1990).

Collection and maintenance of populations. Wild flies were collected over banana baits and maintained in the laboratory in 75 × 25 mm vials on the cactus–yeast–agar medium described in Starmer & Barker (1986). To maintain a large effective population size, random pair matings between progeny from each of a large number of wild

females (100–200) were made and cultured under uncrowded conditions. In the following F_2 generation, progeny from these pair matings were collected as virgins and crossed at random to progeny from other lines. Thus, inbreeding was minimized.

Characters studied. Measurements were made on anaesthetized flies placed on a stage described in Robertson & Reeve (1953). A binocular microscope with a digital filar eyepiece (LASICO) which logged measurements directly to a microcomputer was used for all measurements. The characters used were: (i) thorax length (TL), measured from the anterior margin of the thorax to the posterior tip of the scutellum, with the fly oriented dorsal side upward; (ii) proximal wing length (WP), measured from the wing base to the intersection of the anterior cross vein with the third longitudinal vein; (iii) distal wing length (WD), measured from the intersection of the anterior cross vein with the third longitudinal vein to the wing margin at the distal end of the third longitudinal vein; (iv) wing length (WL), the sum of WP and WD; (v) wing length to thorax length ratio (W/T), WL/TL.

Experimental design. Flies from the F_2 generations used in Thomas & Barker (1993) were used to provide eggs for these experiments. For the Hemmant and O'Hara sites, these adults were placed overnight at 25°C in population cages containing petri plates with live yeast paste as an oviposition substrate. Eggs were washed from the yeast paste and placed on cactus food plates for hatching. Early first instar larvae were transferred to shell vials containing 10 ml of cactus food with a drop of live baker's yeast suspension on the surface. Two densities were used: low, 30 larvae/vial, and medium, 90 larvae/vial. Four replicates of each density were placed at 13, 18, 25 and 29°C, with a 12 h light:12 h dark cycle and 70% relative humidity. These temperatures were chosen to span the range at which *D.buzzatii* is capable of surviving and reproducing during continuous exposure (Watt, 1987). Insufficient larvae were obtained to complete the fourth medium density replicate for Hemmant. Emerging adults were removed from the vials and held on laboratory media until they were measured.

The Trinkey experiment was done at a later date using two replicates at three densities and the same temperatures as for the other sites. The additional density treatment, designated 'high', had 180 larvae/vial. Eggs for this experiment were collected over a 6 h period to better estimate developmental time. Eclosing adults were removed from the vials at a set time daily. They were held on laboratory media until counted and measured.

Analyses. Measurements were log transformed to ensure independence of means and variances (see Thomas & Barker, 1993, for details). Analyses of variance were carried out for log(TL), log(WL), and [log(WL) – log(TL)] (henceforth referred to as log(W/T)) to test the significance of temperature, density, sex, replicate, and their two-way interactions. Linear regressions of log(W/T) on temperature were computed for each site and sex, which were then used for predicting temperature of larval development from the log(W/T) of wild-caught flies. Second degree polynomial regressions of log(TL) on temperature were calculated for the low density treatment within each site for each sex. Regression equations from these analyses were used to predict the log(TL) of wild caught flies, assuming optimal nutrition.

For the Trinkey site, additional data were collected. Median developmental time and viability (defined as survival from early first instar to eclosion) were calculated for each temperature and density combination. Daily collections were not made on four occasions, each no longer than 2 days in length, during the period of eclosion. All of these periods occurred during the prolonged eclosion at 18°C. Individuals emerging during these periods were uniformly apportioned among the missed days for analysis.

Seasonal collections. Collections of wild flies were attempted every 2 months at Trinkey and O'Hara from November 1985 to December 1986. As a result of vagaries of the weather on collecting dates, the series of collections from O'Hara was more complete than that from Trinkey. Size of collection permitting, 100 flies of each sex from each site were measured. For smaller collections all individuals were measured. Rotting cactus cladodes were returned to the laboratory on several occasions and maintained separately at 25°C to rear out adult flies. Watt (1987) found the pupal period of *D.buzzatii* at 25°C to be 5.7 ± 0.4 days. Individuals judged to have pupated in nature were measured. One-way ANOVA's of log(TL) and log(W/T) between rots within sites were calculated.

Results

Laboratory experiments

Tables 1 and 2 give developmental time and viability at each of the treatment combinations for the Trinkey population. These results show that the density treatments were effective in altering the level of nutrition available to larvae. Table 3 presents the ANOVA on the experimental test of the effects of temperature, larval density, sex and their two-way interactions on wing and thorax length measurements and on the ratio of the two measurements. For the linear measures all main effects were highly significant. Fig. 1 shows the effects of temperature and density on thorax length, using the O'Hara population as an example. A second order polynomial regression was fitted

Table 1. Median developmental time in days at various temperatures and densities for Trinkey flies.

	Temperature (°C)		
Density	18	25	29
Low	25.5	13.0	12.0
Medium	28.0	14.0	13.0
High	39.0	15.8	14.5

Low: 30 larvae/10 ml food; Medium: 90 larvae/10 ml food; High: 180 larvae/10 ml food.

Table 2. Viability of Trinkey flies at various temperatures and densities. Viability is measured from early first instar to eclosion.

	Temperature (°C)		
Density	18	25	29
Low	0.60	0.72	0.62
Medium	0.35	0.50	0.44
High	0.17	0.31	0.46

Low: 30 larvae/10 ml food; Medium: 90 larvae/10 ml food; High: 180 larvae/10 ml food.

to these data to provide an equation relating body size (log(TL)) to temperature of larval development at optimal larval nutrition (the low density treatment). The equation for O'Hara females at low density was.

$$\log(TL) = 2.97736 + 0.012436T - 0.000345T^2 \qquad (1)$$

where T is temperature during larval development. The other two populations are qualitatively very similar. Genetic differences between populations for these characters (Thomas & Barker, 1993) make it necessary to produce regressions for each population separately.

Fig. 2 shows the larger effect of temperature changes on wing length relative to thorax length, using the Trinkey population as an example because the additional high density treatment in this experiment makes the effect more obvious. The approximately equal slopes of the lines joining density treatments within temperatures shows graphically the dependence of log(W/T) on temperature. For the ratio of wing length to thorax length (actually the difference of their logs) to be useful as a measure of temperature of larval development, it must be insensitive to the effects of density and interactions betwen density (larval food supply) and temperature. Table 3 shows that for log(W/T) the effect of temperature is highly significant while the effects of density and sex are slight, though sometimes significant, to nonexistent. In no population does either factor account for more than 5% of that amount of variance accounted for by temperature. Similarly, in all three populations, the temperature by density interaction for log(W/T) accounts for less than 1% as much variance as does temperature (Table 3).

For log(W/T) to be useful for interpreting phenotypic variation in nature it would be convenient if the relationship between log(W/T) and temperature was linear. Fig. 3 shows the regression of log(W/T) on temperature for the low density treatment, using O'Hara females as an example. The equation for O'Hara females, rearranged for the prediction of temperature from an observed log(W/T), is:

$$T = [\log(W/T) - 355.153]/(-2.360), \qquad (2)$$

Table 3. Effects of temperature of development, larval density, and sex on adult size and shape measures.

		Hemmant		O'Hara		Trinkey	
Measure	Source	df	MS	df	MS	df	MS
log(W/T)	Temperature	3	35500.5***	3	43599.8***	2	16806.1***
	Density	1	597.2*	1	397.9*	2	781.2***
	Sex	1	46.6	1	189.4	1	679.9**
	Temp. by dens.	3	183.3	3	301.2*	4	75.0
	Temp. by sex	3	235.3	3	699.9***	2	72.4
	Dens. by sex	1	24.5	1	36.8	2	0.2
	Error	458	104.6	553	99.5	550	83.6
log(TL)	Temperature	3	37589.7***	3	41055.3***	2	45830.8***
	Density	1	20971.3***	1	19661.8***	2	80391.0***
	Sex	1	113575.0***	1	146620.4***	1	141907.6***
	Temp. by dens.	3	592.4*	3	244.5	4	2980.0***
	Temp. by sex	3	66.5**	3	613.3**	2	262.0
	Dens. by sex	1	230.0	1	124.3	2	236.5
	Error	458	172.0	553	152.5	550	208.8
log(WL)	Temperature	3	131056.6***	3	159932.4***	2	118139.5***
	Density	1	14490.8***	1	14465.9***	2	65472.9***
	Sex	1	109020.6***	1	136271.0***	1	122941.8**
	Temp. by dens.	3	243.0	3	133.2	4	2981.0***
	Temp. by sex	3	1834.0***	3	2039.6***	2	581.1*
	Dens. by sex	1	104.5	1	296.3	2	222.3
	Error	458	175.0	553	92.6	550	169.2

* $0.01 < P \leq 0.05$, ** $0.001 < P \leq 0.01$, *** $P \leq 0.001$.

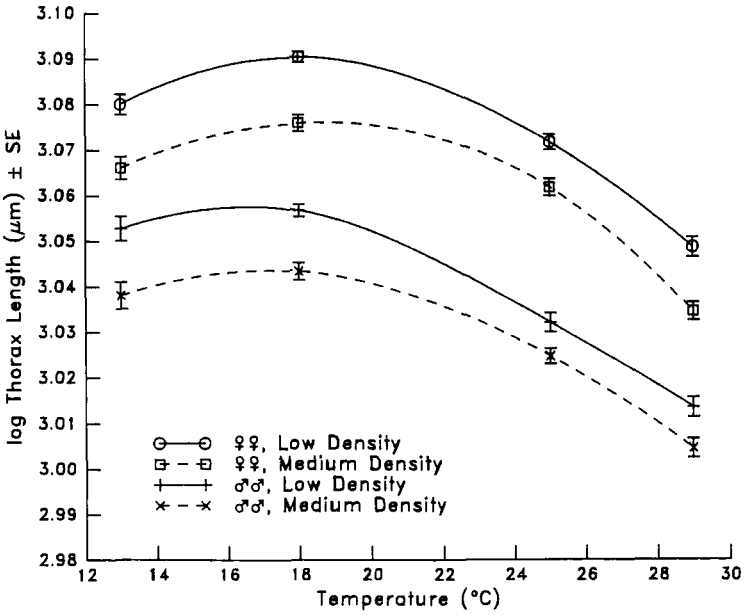

Fig. 1. Effects of temperature during larval development on thorax length for flies from O'Hara reared at different densities. Curves are tensioned splines which approximate the 2° polynomial regressions.

Field experiments

The regression of log(W/T) on temperature of larval development allows the estimation of temperature of larval development from the ratio of simple linear measurements of wing length and thorax length. Once the temperature of larval development has been estimated, the regression of log(TL) on temperature (e.g. equation 1) may be used to estimate potential fly size if each individual had access to optimal nutrition at that temperature. The difference between this predicted size and the observed size is a measure of the nutritional stress experienced by larvae.

The variances of the different measures from the laboratory experiments and the field collections are shown in Table 4. Two points should be noted: (1) the variance in thorax length of the wild-caught flies is very much greater than that of the laboratory reared flies, and (2) the variance of log(W/T) from the two sources are much less variable.

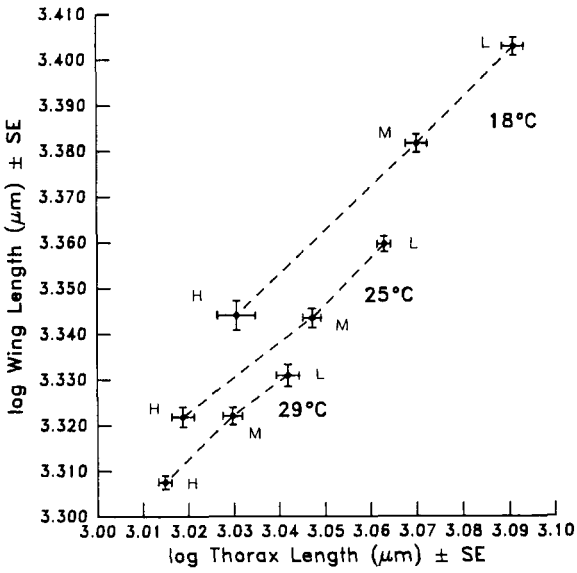

Fig. 2. Plot of wing length against thorax length for the Trinkey population females reared at different temperatures and densities. The lines connecting the data points from the different temperatures are intended only as a visual aid.

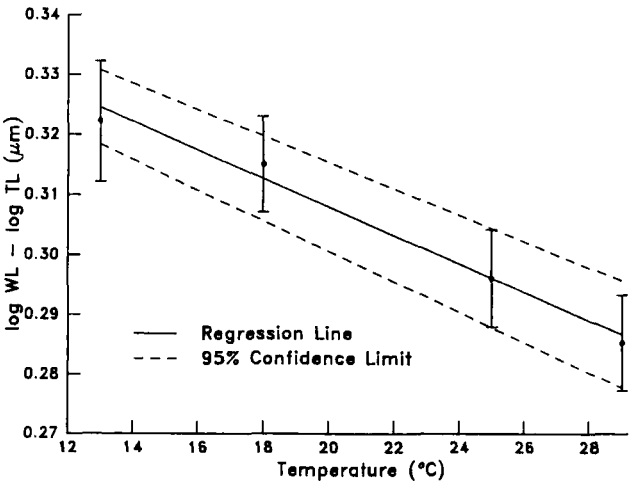

Fig. 3. Linear regression of log(W/T) on temperature during juvenile development from the low density treatment for females from the O'Hara population.

Table 4. Variance ($\times 10^6$) of wild flies and those reared at 18°C. Magnitude of variances of flies reared at 25°C are similar to those reared at 18°C. (μm, log scale).

		Thorax length			Wing length			Wing:thorax		
		Hem.	O'H.	Tri.	Hem.	O'H.	Tri.	Hem.	O'H.	Tri.
Females										
Wild	$s^2 \times 10^6$	817	682	456	678	555	395	186	109	92
	d.f.	140	132	98	137	131	98	137	131	98
Lab (i)	$s^2 \times 10^6$	55	60	57	61	64	61	72	64	69
	d.f.	22	115	134	22	115	134	22	115	134
Lab (ii)	$s^2 \times 10^6$	85	70	–	136	67	–	120	55	–
	d.f.	115	181	–	115	181	–	115	181	–
Males										
Wild	$s^2 \times 10^6$	916	486	663	634	404	430	229	156	148
	d.f.	138	135	99	132	133	99	132	133	99
Lab (i)	$s^2 \times 10^6$	55	73	103	66	69	87	63	103	93
	d.f.	44	128	146	44	128	146	44	128	146
Lab (ii)	$s^2 \times 10^6$	73	100	–	56	77	–	83	49	–
	d.f.	86	181	–	86	181	–	86	181	–

(i) and (ii) refer to independent tests within the populations.

This implies variation in larval diet rather than temperature determines most of the observed natural variation in body size.

The results of applying the regressions from laboratory measurements to collections of field-caught flies are shown in Fig. 4. The predicted temperature of larval development tracks the environmental temperature throughout the year and thorax length increased with decreasing temperatures as expected. Most importantly, throughout the year there was a substantial difference between the observed size of wild flies and their size predicted from the estimated temperature of larval development. This indicates that wild flies seldom, if ever, receive optimal nutrition.

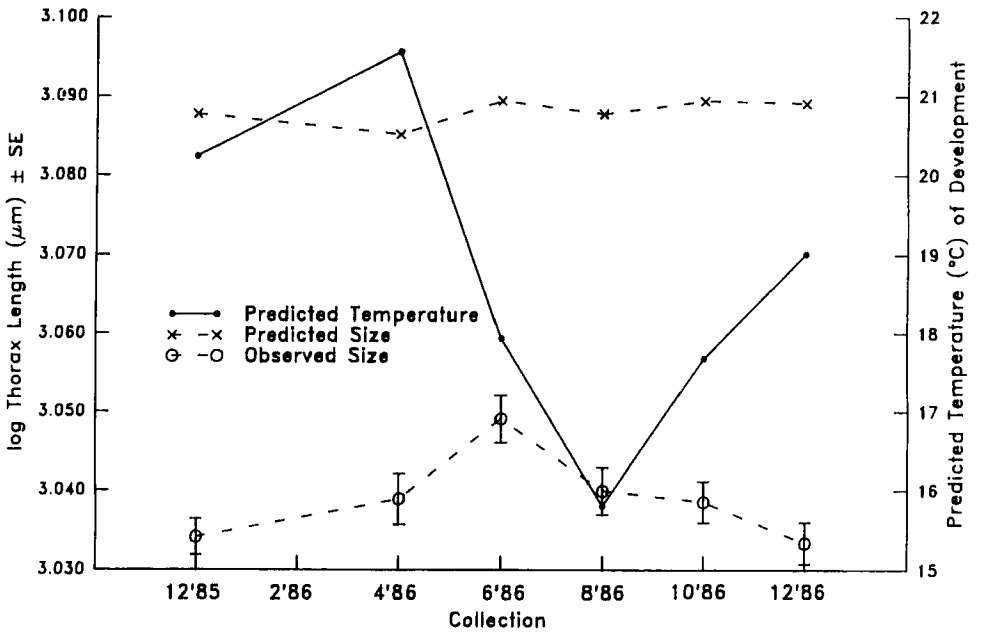

Fig. 4. Predicted temperature of larval development and predicted thorax length assuming optimal nutrition based on measurements of seasonal collections of females from the O'Hara population. See text for further explanation.

Breeding substrate collections

On several occasions, rotting cactus cladodes containing pupae were returned from the field and the adults were allowed to emerge. Adult body size [measured by log(TL)] is determined primarily in the third larval instar (Misra & Reeve, 1964) while wing length is partially determined also during the pupal period (Starmer & Wolf, 1989). Flies emerging from rots were judged to have spent at least the first 2–3 days of their pupal period in the field. After transfer of rots to the laboratory, all flies were subjected to uniform conditions which would tend to even out the variation among individuals emerging from different rots. Simple one-way ANOVA's on log(TL) between rots within sites showed significant variation ($P < 0.05$) in body size among individuals emerging from different rots, while ANOVA's on log(W/T) among rots showed no such variation ($P > 0.05$). The overall means from rot emergences were not significantly different from the means of the corresponding seasonal collections of baited flies, implying the rots are good samples of the populations at large. These results suggest temperature of larval development is fairly homogeneous across a site but that the quality of nutrition available to larvae in different rots varies substantially.

Discussion

Robertson's (1987) suggestion that the ratio of wing length to thorax length in *Drosophila* is largely independent of the level of nutrition supplied to larvae has been borne out by this work. This observation, coupled with the known dependence of this ratio on temperature of larval and pupal development and the dependence of adult body size on nutrition supplied to third instar larvae, provides a reliable method for separating the effects of temperature and resource availability on adult body size in natural populations. Simple linear measurements of wild-caught flies, coupled with laboratory derived regression equations, allow analyses of resource availability at a fine-scale across a habitat over time.

An independent attempt was made to verify the results from the laboratory regression-based work by using the temperature dependence of the rate of hydrolysis of sucrose solutions (results not shown) (Berthet, 1960). Small quantities of sucrose solution in vials were placed inside a sample of *in situ* breeding substrates (rotting cactus cladodes) at Trinkey for a set time prior to a seasonal collection of adult flies from that site. Integrated temperature estimates were derived from measurements of the amount of sucrose converted to fructose and glucose. The observed variation in thorax length from the contemporaneous seasonal collection was more than twice that expected over the temperature range estimated with the sucrose inversion technique, assuming uniform levels of available nutrition across the rots. This result corroborated that from the measurements of flies emerging from the rots.

Could fluctuations of temperature in natural environments mislead us when applying constant temperature laboratory regressions to the field? This question is best split into two questions, considering separately the situations where mean temperature throughout larval development is constant and where it is not. The method presented in this paper does depend on a high serial correlation in temperature over the period of larval development, particularly during the later stages of development. F. W. Robertson (personal communication) has preliminary data which suggest that the critical size (Robertson, 1963) in larval growth is positively correlated with temperature. Therefore, if temperature during early larval life differed substantially from that during the pupal period we could be misled. In the natural populations examined here this does not seem likely to be a serious problem. On the time scale of larval life, most variation in temperature is diurnal and the mean temperature tomorrow is likely to be close to that of today. The importance of temperature variation about a constant mean depends on the period of fluctuation and the lengths of time in which thorax length and wing length are determined. As already mentioned, most temperature variation in nature is on a diurnal time scale. Thorax length is determined over a period of days during larval growth, particularly during the third instar (Robertson, 1963). Wing length is largely determined during a period of several days during pupal development (Starmer & Wolf, 1989). Thus one would expect that temperature fluctuations would not affect strongly the results of this method. Our field collections of flies necessarily represent an average of flies developing over a period of time and this should tend to dampen any such effects of fluctuation of temperature about a mean. However, it should be kept in mind in working in other systems.

T. Prout (personal communication) has pointed to a potential difficulty with the transformation used here and has suggested a more general alternative. Fig. 2 shows that temperature has the effect of changing the y-intercept of the curves relating wing and thorax lengths. Unless the slope of this relationship is equal to 1 the measure used here (log wing length − log thorax length) is contaminated by temperature. For the data presented here this slope is approximately 0.86, so the results are not strongly influenced by this effect. Prout suggests W/T^B as an alternative to W/T, where B is the slope and W and T are the wing and thorax lengths, respectively. This transformation should be used in systems where the slope is significantly different from 1.

Atkinson (1979) has used a multiple regression approach to investigate the effects of temperature and larval competition on adult body size of domestic *Drosophila*. He found that larval density accounted for a significant amount of variation in body size of adult *D.melanogaster* and he interpreted this as evidence of larval competition. This method requires the measurement of larval densities from field substrates and the estimation of the quality of those substrates by some other means. Atkinson (1979) admits that density by itself may be a poor estimator of larval food supply and he attempted to improve on this by

carrying out separate multiple regression analyses for different breeding sites (i.e. apples and tomatoes). The method described in this paper eliminates the need for direct measures of larval food supplies in the field, and in fact permits the estimation of the effect of variation in larval food supply at a fine scale with minimal manipulation of the field site, simply by enclosing particular substrates and removing adults as they enclose.

Jones *et al.* (1987) investigated the thermal niche of *Drosophila melanogaster* by measuring the expressivity of a temperature-sensitive eye colour mutation in the descendants of flies released into the wild. Their results suggest that behavioural responses of the flies act to limit exposure to extremes of temperature. The method reported here has several advantages over the mutant release method of Jones *et al.* (1987): (1) it is natural, requiring no introduction of alien flies, (2) it works on *Drosophila* species for which there are not the appropriate mutants, and (3) it gives important additional information about the nutritional status of larvae. It is also possible that this method may work with other insect groups.

Flies from the populations reported on here were nutritionally stressed throughout the year, despite large variations in population size and available quantity of breeding substrate as a consequence of seasonal climatic changes and the bivoltine lifecycle of the *Cactoblastis* moth which initiates most of the rotting of cactus cladodes (Barker & Mulley, 1976). Most of the observed variation in body size is due to variation in the nutrition provided by individual rots, rather than variation in temperature among rots. It would be interesting to know if this variation resulting from resource availability is mitigated by habitat selection by the adults colonizing individual rots.

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