

Latitudinal clines for morphometrical traits in *Drosophila kikkawai*: a study of natural populations from the Indian subcontinent

DEV KARAN¹, ASHOK K. MUNJAL¹, PATRICIA GIBERT², BRIGITTE MORETEAU², RAVI PARKASH¹ AND JEAN R. DAVID^{2*}

¹Department of Biosciences, Maharshi Dayanand University, Rohtak 124001, India

²Laboratoire Populations, Génétique, Evolution, CNRS 91198 Gif sur Yvette Cedex, France

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Summary

Natural populations of *Drosophila kikkawai* were collected in India and Sri Lanka, along a latitudinal transect ranging from 6.8° to 31.8° N latitude. Six morphometrical traits were analysed: wing and thorax length, body weight, ovariole number, and abdominal and sternopleural bristle numbers. Significant clines were observed for the three size-related traits and for ovariole number, corresponding to a regular increase in the mean value with latitude, but not for bristle numbers. Due to the utilization of two types of laboratory food, data were distributed into two separate data sets. A low-nutrient food produced smaller flies on average because of more intense crowding. The two rearing conditions produced significant clines but with significantly different slopes. The wing/thorax ratio, which is inversely related to wing loading, also increased with latitude. The analysis of Indian climatic conditions suggested that winter temperature, decreasing from south to north, could be more efficient than summer temperature, which varies in an opposite way, as a selective factor for inducing the clinal variations. The sibling species *D. leontia*, which is known only from the humid tropics, was found to be much smaller than *D. kikkawai* and did not fit the clinal regressions. Such morphological differences should help to identify the two species when found in sympatry.

1. Introduction

Genetic variation between geographic populations is a common feature in many *Drosophila* species. When variations can be correlated with an environmental gradient, an adaptive significance is generally assumed, and in this respect latitudinal clines are of special interest (Endler, 1977; Merrel, 1981; David & Capy, 1988). In several *Drosophila* species, body size has been shown to increase with latitude, that is when moving from warmer to colder places. Such clinal variations have been described in the American *D. robusta* (Stalker & Carson, 1947, 1948), in the European *D. subobscura* (Prevosti, 1955; Misra & Reeve, 1964) and in several cosmopolitan species such as *D. melanogaster* and *D. simulans* (Tantawy & Mallah, 1961; David & Bocquet, 1975*a, b*; Coyne & Beecham, 1987; Capy *et al.*, 1993; James *et al.*, 1995, 1997; Azevedo *et al.*, 1996) and *D. virilis* (David &

Kitagawa, 1982). In all these cases, average temperature has been considered to be the responsible selective factor, even if we still do not know why it may be better, for an ectotherm, to be bigger in a cold climate (Powell, 1974; David *et al.*, 1994; Partridge *et al.*, 1994).

D. kikkawai is a cosmopolitan species, included in the *melanogaster* group (*montium* subgroup) of *Sophophora* subgenus. It is native to Southern Asia but has been introduced in other parts of the world, especially South America from where it was described (David & Tsacas, 1981; Lemeunier *et al.*, 1986). *D. kikkawai* is typically a tropical and subtropical species, which is unable to colonize cold regions and is not found above latitudes of 35°. It exhibits some interesting genetic features such as a single locus polymorphism for abdomen pigmentation (Freire-Maia, 1964) and complex structural polymorphisms of the Y and fourth chromosomes (Baimai *et al.*, 1986).

For studying climatic adaptations in tropical species, the Indian subcontinent is an especially

* Corresponding author. Tel: 1 69 82 37 13. Fax: 1 69 07 04 21. e-mail: david@pge.cnrs-gif.fr

Table 1. *Female morphometrical traits of the first data set, obtained in Rohtak with a low-nutrient medium and somewhat crowded larval conditions*

Locality	Latitude (°N)	Thorax length	Wing length	Wing/thorax ratio	Body weight	Sternopleural bristles	Abdominal bristles	Ovariole number
Colombo	6.8	87.78	199.19	2.269	0.79	14.30	43.84	30.95
Bangalore	12.6	90.63	216.68	2.394	0.79	12.82	41.46	35.85
Hyderabad	17.2	97.09	236.08	2.434	0.83	16.76	46.12	30.70
Nagpur	21.1	96.36	239.81	2.494	0.94	15.70	49.54	36.35
Bhopal	23.2	98.93	240.70	2.434	0.95	14.62	45.46	30.85
Agra	27.1	98.93	241.49	2.442	0.99	16.34	47.98	36.20
Rohtak	28.9	100.65	263.80	2.623	1.04	13.62	50.66	37.50
Solan	30.8	104.02	277.40	2.674	0.98	15.18	48.74	41.05
Shimla	31.3	105.04	262.28	2.499	1.12	13.96	47.84	38.55
Standard error range		0.40–0.63	0.65–1.22	0.008–0.020	0.009–0.099	0.26–0.39	0.43–0.73	0.36–0.75
Overall mean		97.71	241.94	2.474	0.94	14.81	46.85	35.33

Thorax and wing length are expressed in millimetres $\times 100$; body weight in milligrams. Mean values are calculated on 50 flies except for ovariole number (20 flies) and body weight (10 groups of 10 flies).

favourable place. From South to North, the seasonal thermal amplitude shows a regular increase, with progressively more marked cold and warm seasons. *D. kikkawai* is quite abundant everywhere in India, and also in Sri Lanka, and we compared populations collected along a latitudinal transect.

In the present paper we investigated genetic variation in morphometrical characteristics: body weight, thorax and wing length, bristle numbers and ovariole number. Well-marked clines were observed for size-related traits, and also for ovariole number.

2. Material and methods

(i) Populations and experiments

A first set of eight populations, collected in 1995 along a latitudinal transect (Bangalore, Hyderabad, Nagpur, Bhopal, Agra, Rohtak, Solan and Shimla) was investigated in Rohtak within 1 month of collection. For each population, a mass culture was established from at least 15 wild-collected females. Other populations were later collected and investigated in France, a few months after collection. A population from Sri Lanka collected near Colombo was investigated both in France and in India, and also a strain (founded by two isofemale lines) of the sibling species *D. leontia* (Tsacas & David, 1977) was measured in both laboratories.

It turned out that some discrepancies existed between Indian and French data, especially for size characters: mean values obtained in the Rohtak laboratory were lower than values obtained in France for the same traits and at the same temperature of 25 °C. Further investigations suggested that this discrepancy was due to differences in larval rearing conditions. In France, larvae were grown on a high-nutrient killed yeast medium (David & Clavel, 1965)

with a larval density of about 100 per vial. In India, larval density was also controlled, but breeding was done on a cornmeal, low-nutrient food, seeded with live yeast, in which crowding effects were more pronounced, so that average size was smaller. It was thus decided to repeat the experiments in India with the same medium as in France. These new experiments were done a year later. Four natural populations could be sampled again (Shimla, Solan, Rohtak and Bhopal). Three other populations (Bangalore, Hyderabad and Nagpur) had been kept in the laboratory for more than a year and were again measured. The Agra population had been lost.

(ii) Traits measured

In all cases flies were grown at 25 °C. For the first investigations made in India, hereafter designated as the 'first data set', both males and females were measured. In later experiments made either in France or in India, and using the killed yeast food (hereafter called the 'second data set'), only females were measured. In the present paper, only female data will be presented for both data sets.

Size was assessed by measuring thorax length in a lateral view from the neck to the tip of the scutellum, with a micrometer in a binocular microscope. Wing length was measured in the same way from the thoracic articulation to the tip. Micrometer data were transformed and expressed in millimetres $\times 100$. For each fly, the wing/thorax ratio was also calculated and considered as a specific trait, as in other papers (Thomas, 1993; David *et al.*, 1994; Pétavy *et al.*, 1997). Body weight was measured on fed females aged 2–3 days. Groups of 10 females were simultaneously weighed and values expressed in milligrams per fly. Bristles were counted either on both sides of the thorax (sternopleural bristles) or on segments 3 plus

Table 2. Female morphometrical traits of the second data set

	Latitude (°N)	Study in	Thorax length	Wing length	Wing/thorax ratio	Body weight	Sternopleural bristles	Abdominal bristles	Ovariole number
<i>D. kikkawai</i>									
Colombo	6.8	France	101.28	222.08	2.193	0.97	12.8	—	30.50
Pondicherry	12.0	France	103.2	228.64	2.216	0.85	14.95	—	33.53
Bangalore	12.6	India	97.16	218.68	2.255	0.94	12.56	41.84	41.70
Hyderabad	17.2	India	102.2	238.42	2.334	1.01	15.56	47.56	30.3
Bhubaneswar	20.2	France	106.85	242.8	2.273	1.12	14.45	—	39.59
Nagpur	21.1	India	104.3	243.74	2.339	1.19	14.84	48.00	39.4
Bhopal	23.2	India	104.02	240.38	2.312	1.23	14.12	41.00	38.8
Delhi	28.4	France	110.07	258.27	2.347	1.31	12.33	—	38.5
Rohtak	28.9	India	108.78	257.18	2.366	1.31	14.68	50.60	39.5
Rohtak	28.9	France	109.36	255.43	2.337	1.31	11.97	—	38.43
Chandigarh	30.4	France	109.13	260.93	2.391	1.32	13.3	—	36.13
Solan	30.8	India	108.5	265.16	2.444	1.32	17.44	50.00	43.4
Shimla	31.3	India	109.76	263.9	2.405	1.36	14.40	51.32	40.7
Standard error range			0.31–1.11	0.76–2.26	0.009–0.021	0.0002–0.055	0.29–0.48	0.66–0.86	0.47–2.40
Overall mean			105.74	245.82	2.324	1.172	14.11	47.19	37.73
<i>D. leontia</i>									
Colombo	6.8	India	78.08	176.22	2.259	0.51	12.22	37.08	23.75
Colombo	6.8	France	78.13	179.73	2.302	0.48	12.17	—	22.57

Measurements were done either in Rohtak (India) or in Gif (France) using a high-nutrient killed yeast food and low larval density. Same conditions as in Table 1.

4 of the abdominal sternites (abdominal bristles). Abdominal bristles were counted only in India. Females aged 4–6 days were dissected under a binocular microscope with tiny insect pins (minuties); ovarioles of both ovaries were counted after fixation in potassium dichromate.

3. Results

Results of the first data set, obtained in Rohtak with a low-nutrient food, are given in Table 1. Similar measurements, corresponding to the second data set, are presented in Table 2 together with the results for *D. leontia*.

Measurements in the second data set were done with the same rearing technique, either in India or in France. In one case (*D. kikkawai*, Rohtak) the same population was investigated in both places, using independent samples. For *D. leontia*, the same strain was measured in both countries. In these two cases, for which precise comparisons can be made, the results were very similar and not significantly different. Also the latitudinal regression analyses, which will be presented in a later section, led to the same conclusion: a general concordance of the measurements made in the two laboratories. There was, however, one

exception concerning sternopleural bristle number, for which average values obtained in France were often lower than in India. For example, values found for Delhi and Chandigarh and measured in France were significantly lower than values obtained for other populations of similar latitudes, such as Bhopal or Solan. We believe this discrepancy is due to the fact that in *D. kikkawai* these bristles are especially fragile and easily broken. The count decreases when adult flies are kept for several days under crowded conditions in laboratory vials.

Data concerning *D. kikkawai* (Tables 1, 2) were submitted to one-way ANOVA (not shown) and a highly significant heterogeneity between populations was evidenced for each trait. As already indicated in Section 2, systematic differences were observed between the first and second data sets, especially for size-related characters such as thorax length and body weight, and also for the wing/thorax ratio. Differences were less pronounced for the numerical traits, bristle and ovariole numbers, which might be less sensitive to crowding effects.

Variations among populations were further investigated in relation to latitude or origin, and regression parameters are given in Tables 3 and 4. In both data sets a significant latitudinal effect was observed for

Table 3. Regression analysis of morphometrical traits according to latitude of origin for the first data set

	Intercept	Slope	<i>r</i>	<i>n</i>
Thorax	83.52 ± 1.521	0.643 ± 0.0647***	0.97	9
Wing	182.34 ± 7.553	2.698 ± 0.3299***	0.95	9
Wing/thorax ratio	2.21 ± 0.069	0.018 ± 0.0029*	0.84	9
Body weight	0.66 ± 0.045	0.012 ± 0.0019***	0.93	9
Sternopleural bristles	14.39 ± 1.344	0.019 ± 0.0572 ns	0.13	9
Abdominal bristles	40.99 ± 1.934	0.265 ± 0.0823*	0.77	9
Ovariole number	28.59 ± 2.777	0.305 ± 0.1182*	0.70	9

r, correlation coefficient; *n*, number of populations. The intercept corresponds to the expected value at the Equator.

Significance level: ns, not significant; * $P < 0.05$; *** $P < 0.001$.

Table 4. Regression analysis of morphometrical traits according to latitude of origin for the second data set

	Intercept	Slope	<i>r</i>	<i>n</i>
Thorax	96.122 ± 1.740	0.426 ± 0.075***	0.88	12
Wing	204.01 ± 3.633	1.868 ± 0.156***	0.97	12
Wing/thorax ratio	2.139 ± 0.026	0.008 ± 0.0011***	0.92	12
Body weight	0.720 ± 0.0494	0.020 ± 0.0021***	0.95	12
Sternopleural bristles	13.439 ± 1.261	0.033 ± 0.0541 ns	0.19	12
Abdominal bristles	37.447 ± 4.498	0.413 ± 0.184 ns	0.71	7
Ovariole number	31.141 ± 3.099	0.291 ± 0.133 ns	0.57	12

r, correlation coefficient; *n*, number of populations. The intercept corresponds to the expected value at the Equator.

Significance level; ns, not significant; * $P < 0.05$; *** $P < 0.001$.

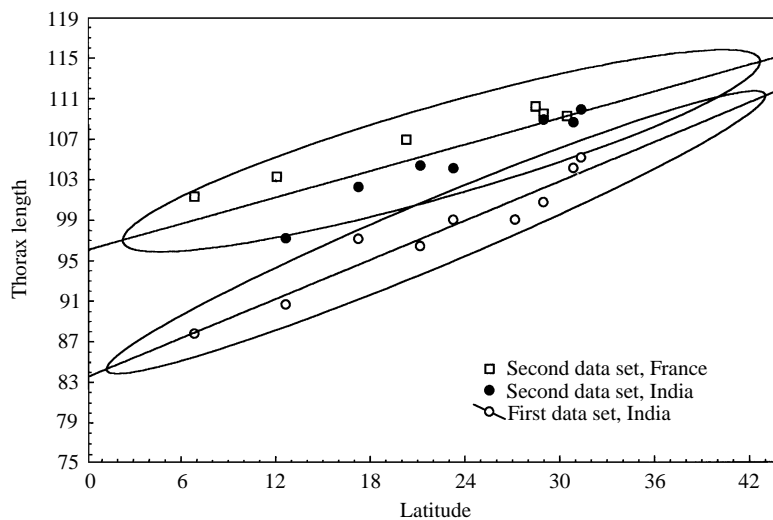


Fig. 1. Increase in thorax length with latitude of origin ($^{\circ}$ N) in the two data sets of *D. kikkawai*. Ellipses of 95% confidence are shown for each data set. Notice the similarity of measurements made in France and India for the second data set, and the significantly different slopes of the linear regressions.

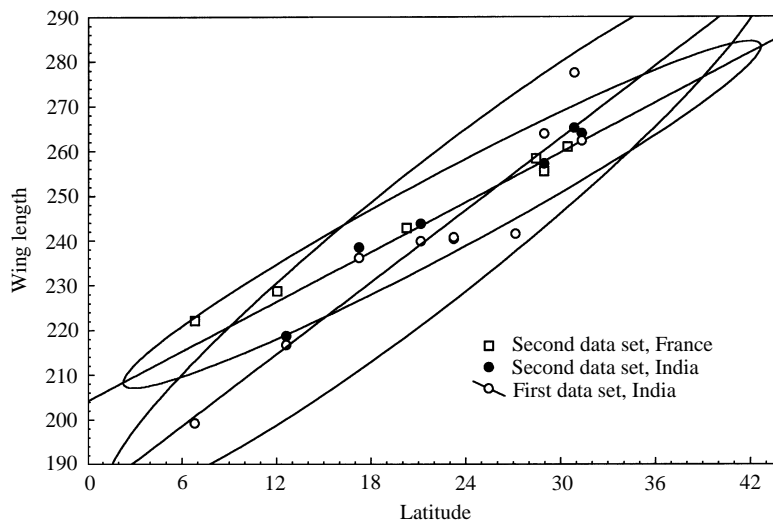


Fig. 2. Increase in wing length with latitude of origin in *D. kikkawai*. Notice the broad overlap of the two data sets, but the different slopes of the regression.

size characters, i.e. an increase in thorax length, wing length, body weight and wing/thorax ratio. For the other traits, results were less clear. Positive slopes were observed in all cases but they were generally not significant, or slightly significant for ovariole number and abdominal bristles in the first data set only.

Latitudinal variations of thorax and wing length are illustrated in Figs. 1 and 2. For thorax length, the two sets of data are separated and included in different confidence ellipses. French and Indian measurements in the second data set are very close and included in the same regression. Thorax length was consistently smaller in the first data set, but also the regression coefficients were significantly different, the slope was steeper and the intercept was smaller in the first data set. For wing length, a broad overlap was observed

between the two sets of data and the overall means were not different. As for thorax length, the slope was steeper in the first data set, but the difference is barely significant ($P = 0.023$).

The wing/thorax ratio (Fig. 3) was consistently higher in the first set of data and the difference between overall means was highly significant ($P = 0.0021$). Again the slope was steeper in the first set but not significantly so ($P = 0.88$). As indicated previously, the difference between the first and second experiments was mainly due to crowding level. These data suggest that wing length was less affected than thorax length by crowding, hence the higher wing/thorax ratio.

Body weight, in females aged 2–3 days, may also be considered as a size character, and it also increased

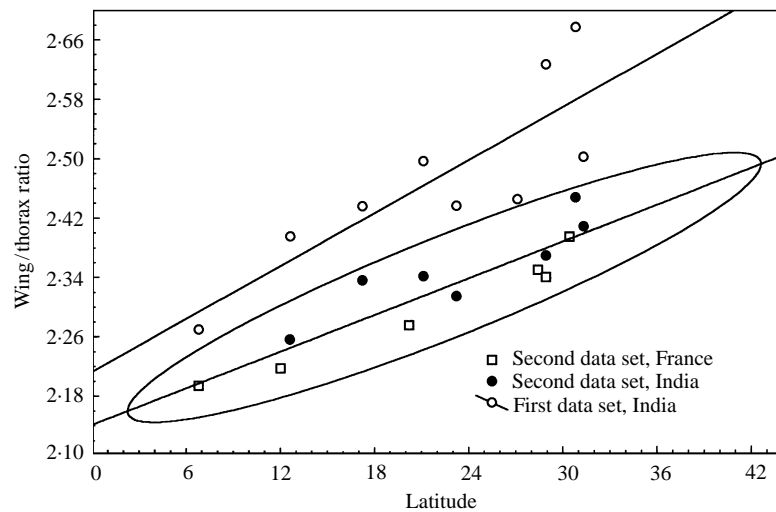


Fig. 3. Increase in the wing/thorax ratio according to latitude of origin in the two data sets of *D. kikkawai*. The 95% confidence ellipse is shown only for the second data set, for clarity.

with latitude. In that case, a major difference was found between the two data sets, with overall means of 0.94 and 1.17 mg. Also the regressions were different, but in that case the slope was smaller in the first data set (0.0124 vs 0.0201; $P = 0.017$).

Ovariole number appeared less affected by experimental conditions since the overall means, 35.3 and 37.7, were not significantly different. The latitudinal effect (an increase in number with latitude) was significant only in the first data set. We pooled all observations (22 different values) into a single sample, and in that case the latitudinal effect became much clearer, with a slope of 0.296 ± 0.087 ($P = 0.003$).

Abdominal bristles exhibited some trend for a latitudinal increase, but only one regression (abdominal bristle in the first data set) was significant. Pooling all abdominal bristle measurements made in India (these bristles were not counted in France) provided a non-significant regression slope (0.158 ± 0.137 , $P = 0.269$). For sternopleural bristles, the whole data set ($n = 22$) also failed to show a significant latitudinal trend: the slope was 0.0218 ± 0.039 ($P = 0.583$).

4. Discussion and conclusion

As stated in Section 1, at least five *Drosophila* species were previously shown to exhibit latitudinal clines for morphometrical traits. These clines generally concerned size-related traits, such as wing and thorax length. To our knowledge, the most detailed investigations were made on *D. melanogaster* and *D. simulans* (Capy *et al.*, 1993, 1994), in which six different traits were simultaneously analysed: body weight, thorax and wing length, sternopleural and abdominal bristle number, and ovariole number. For each trait in each species, a significant increase with latitude was observed. The same traits were measured in *D.*

kikkawai, and significant latitudinal increases have been found for four of them, but not for bristle numbers. A possible explanation for the discrepancy is that, for *D. melanogaster* and *D. simulans*, broader latitudinal ranges could be considered, from the Equator up to about 50° latitude, since both species can proliferate under temperate climates. For *D. kikkawai*, we considered a narrower latitudinal range (from 6.8° up to 31.3° N) corresponding only to tropical and subtropical climates. A broader latitudinal range is likely to increase the probability of obtaining a significant latitudinal effect. This might explain why bristle numbers did not exhibit a significant cline in *D. kikkawai*. However, it also remains possible that, in *D. kikkawai*, these traits do not respond to climatic changes, since their possible adaptive significance is not understood (Coyne & Beecham, 1987; Capy *et al.*, 1993).

In trying to explain latitudinal clines, investigators have generally considered temperature as a key environmental factor. When a broad latitudinal range is considered, for example from 0 to 50 the correlation between latitude and average year temperature exceeds 0.9 (Capy *et al.*, 1993). The likely selective role of temperature, at least for size characters, is enforced by laboratory experiments in various species: keeping strains at different temperatures induced, in the long term, divergent genetic sizes, that is bigger flies at low temperature and smaller ones at high temperature (Anderson, 1973; Powell, 1974; Cavicchi *et al.*, 1985; Partridge *et al.*, 1994).

In the case of natural populations of *D. kikkawai*, the role of average temperature is not so clear. Populations investigated, from Colombo to Rohtak, were collected at low altitudes, while Solan and Shimla populations were collected at altitudes of 1250 and 2000 m respectively. Between Colombo and Rohtak, average year temperature exhibits only a

slight and non-significant latitudinal decrease, from 27 to 25 °C. If we exclude the Solan and Shimla populations from calculations, the latitudinal clines observed for size traits remain highly significant. What distinguishes southern and northern low-altitude localities is the seasonal thermal amplitude. Southern places are characterized by a mild winter; going North the winter becomes progressively colder, while the summer becomes progressively hotter and drier. The latitudinal cline that was described here correlates both with colder winters and with warmer summers. Summer conditions are probably responsible for a latitudinal cline of increasing desiccation tolerance, which has been observed in three other species (*D. ananassae*, *D. melanogaster* and *Z. indianus*) (unpublished observations). In the case of *D. kikkawai*, hot summers might be expected to induce a latitudinal decrease in size, which was obviously not the case. Natural population data suggest that, if climatic variables are responsible for the cline, winter conditions should be prevalent over summer conditions, that is adaptation to cold should be more powerful than adaptation to heat. Finally, for the cline in ovariole number, it may be possible that, as argued by Capy *et al.* (1993), populations living under greater seasonal variations are more *r*-selected, i.e. exhibit a higher potential for proliferation. Another possible interpretation is based on resource allocation. Populations of various species, including *D. melanogaster*, exhibit a better starvation tolerance when living near the Equator (Da Lage *et al.*, 1990 and unpublished observations), which is presumably due to a higher lipid content (Van Herwege & David, 1997). In other words, populations from the humid tropics seem to allocate more resources to survival but less to reproduction, and hence the lower ovariole number.

It might be interesting in species that exhibit similar latitudinal effects to compare the steepness of the clines, that is the genetic reactivity to temperature. Such comparisons are difficult, however, because different experimental techniques were often used by different investigators. The importance of experimental conditions was clearly demonstrated in the present study. Latitudinal trends were established in the two sets of data, which used either a low- or a high-nutrient food medium. If the results were qualitatively similar, they were quantitatively different for size-related traits. As expected, the use of a low-nutrient food, amplifying crowding effects, produced smaller flies, i.e. shorter thorax and lower body weight. What was unexpected, however, and deserves further investigation, is the fact that the slopes of the clines were not the same. Curiously, slopes in the first data set were higher for thorax and wing length but smaller for body weight.

The wing/thorax ratio was on average higher in the first data set, suggesting that wing length is relatively

less susceptible to rearing conditions than body size. The augmentation of the wing/thorax ratio with latitude arises from the fact that wing length changed relatively more than thorax length. Wing/thorax ratio is negatively correlated with wing loading, and a lower wing loading, due either to genetic polymorphism or to phenotypic plasticity, may be considered as an adaptation for flying in a colder environment (Stalker, 1980; Pétavy *et al.*, 1997). It is interesting to point out that the genetic changes observed here are parallel to the epigenetic changes related to growth temperature.

A last observation concerns the sibling species *D. leontia*, which was already known from Singapore, Malaysia, Thailand, Burma and southern India (Baimai *et al.*, 1986). From a morphological point of view, this species is difficult to distinguish from *D. kikkawai* (Tsacas & David, 1977), but interspecific crosses produce sterile F₁ males and fully fertile females (David *et al.*, 1978). Our data show that it is a much smaller species than *D. kikkawai*, with also a much lower ovariole number but a higher wing/thorax ratio. Plotted against the latitudinal variations observed in *D. kikkawai*, values of *D. leontia* are clearly separate. These morphometrical differences should help discriminate the two species when they are living in sympatry.

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