

BIOMETRICAL EFFECTS OF THE INVERSION POLYMORPHISM OF *KIEFFERULUS INTERTINCTUS* (SKUSE)

By JON MARTIN*

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Abstract

The Lonsdale, Barwon, and Corio inversions of *K. intertinctus* produce biometrical effects in larvae. The *Lo*, *Ba*, and *Co* sequences all increase the length of the ventral tubules in both male and female larvae. This effect may help in understanding the basis of the non-random association between the Lonsdale and Barwon inversions. *Lo* and *Ba*, whose occurrence together is advantageous, are more common in warmer regions where the oxygen content of the water is low. Larger ventral tubules provide an increased area for respiratory exchange.

In addition, the inversions probably also affect larval length. The *Lo*, *Ba*, and *St^{Co}* sequences apparently decrease the length of female larvae but increase the length of male larvae.

I. INTRODUCTION

Although numerous cases of inversion polymorphism have been reported in a great variety of animals, particularly insect species, there have been very few cases where these polymorphisms have been further investigated to determine how they confer advantage on their carriers. Some species of *Drosophila*, notably *D. pseudoobscura*, have been shown to exhibit a variety of physiological differences between the carriers of different inversion sequences (for reviews see da Cunha 1960; Parsons and McKenzie 1972). At least in the case of the Standard, Chiricahua, and Arrowhead sequences of *D. pseudoobscura* there are also biometrical effects. The *ST/CH* is heaviest, and therefore presumably largest, followed by *CH/CH*, with *ST/ST* being the smallest and lightest karyotype (Thomson 1971), while *AR/AR* is larger and heavier than *AR/CH* or *CH/CH* (Tantawy 1961). Ebitani (1971) found that in *D. ananassae* the heterozygotes for inversion *In 2L* were heavier than either homozygote at all stages of development for almost all the experimental conditions he tested.

Amongst the Chironomidae the only case previously studied was in *Chironomus nuditarsis* (Rosin and Fischer 1968). Here the effect of alternate sequences present in chromosome arms A and B on body size, as reflected by width of the head capsule of the larva, was investigated. For both arms, the inversion homozygotes were significantly smaller than the normal homozygotes and the heterozygotes. The heterozygotes did not differ significantly in size from the normal homozygote.

* Genetics Department, University of Melbourne, Parkville, Vic. 3052.

The only other instance in which biometrical effects of inversions appears to have been investigated is in the small wingless grasshopper, *Keyacris scurra* (White and Andrew 1959, 1960; White *et al.* 1963). Here there are two interacting pericentric inversion systems, the Blundell inversion in the *CD* chromosome and the Tidbinbilla inversion in the *EF* chromosome. The standard sequence of each inversion has been shown to be size-increasing, while the Blundell and Tidbinbilla sequences are size-decreasing.

In the course of cytological studies on the inversion systems of the midge *Kiefferulus intertinctus* (Skuse), previously called *Chironomus intertinctus* (Martin 1962, 1963, 1965), measurements were made on the fixed larval bodies before dissection to determine whether the inversions have any effect on the size of the larva carrying them.

II. MATERIAL AND METHODS

The material utilized in this study came from the localities previously listed by Martin (1962, 1963, 1965). Fixed larvae were used for measurements because they are easier to handle and are almost straight, requiring very little manipulation to obtain an accurate measurement. All measurements were made under the dissecting microscope using an eyepiece micrometer. Larval length was measured at $\times 6$ magnification, to the nearest micrometer unit, which represents measurement to slightly less than the nearest 0.2 mm. Ventral tubule length was measured to the nearest micrometer unit at $\times 25$ magnification, which represents measurement to the nearest 0.04 mm. As pointed out by Martin (1963), the length of fixed larvae is about 20% greater than that of living larvae, but this difference should be consistent in all specimens. Late prepupae, or larvae which were obviously damaged and therefore not fully extended, were not utilized for measurement.

The major problem in this study was that the various samples were too small to show any but the most extreme effects, and the results from different samples could not be pooled because of the seasonal variations in size which occur (Martin 1963). Therefore a non-parametric test suggested by Dr. R. E. Blackith (now of Trinity College, Dublin) was used. This test was as follows: the sex, karyotype, and measurements of each larva were recorded on a separate card. The cards for each collection were taken, separated into males and females, then each sex was further sorted into the karyotype of the inversion under study, e.g. St/St, St/Inv, Inv/Inv, where St and Inv designate the particular standard and inversion sequences respectively. Three pairwise comparisons of these karyotypes were possible, viz.: St/St and St/Inv; St/Inv and Inv/Inv; St/St and Inv/Inv. The cards in each pile were shuffled, a card from each pile chosen and compared, and the larger karyotype recorded. The limit to the number of comparisons which could be made was the number of the least frequent karyotype in each pair. Thus, if the inversion frequencies differed greatly, only a few comparisons could be made involving the rare sequence, but each individual had an equal chance of being used for a comparison. Although this meant that a percentage of the data had to be wasted, it had the advantage that the results from different collections could be pooled, if the assumption was made that the different karyotypes would show similar size relationships at different seasons and at different localities. While the first assumption may be valid, there is no *a priori* reason to suppose that the latter assumption is valid. In the grasshopper *Keyacris scurra*, however, the populations studied showed similar relationships even though the populations were geographically remote (White and Andrew 1960), except when the population showed anomalous inversion frequencies (White *et al.* 1963).

The basis of the test, then, was that if the karyotype had no influence on the size of the larva, each pairwise comparison would show no significant deviation from a 1 : 1 ratio. If, however, one sequence is size-increasing then karyotypes involving this sequence would be expected to be larger and so the 1 : 1 ratio would not be achieved.

Since it has been shown that there is a marked non-random association between the Lonsdale and Barwon inversions (Martin 1965), comparisons were also made to determine the effect of the combined *Lonsdale* and *Barwon* karyotypes. Only five of the nine different karyotypes were com-

pared, the four double homozygous classes and the double heterozygotes, as these seemed to be the most important in the association. Each of these was compared with the other four karyotypes, making 10 comparisons in all.

The analysis was performed twice, with two differences between the two runs. In a first analysis (Martin 1966), those cases in which the two individuals compared were of equal size were ignored. In the analysis reported here these cases were recorded and were then divided equally between the two classes before the ratio was tested by a χ^2 test. Also, in this analysis, Yates' correction was applied to the χ^2 tests. The results of the two analyses were very similar, but there were fewer significant results in the second analysis due to the altered method of testing.

III. RESULTS

The results of the analysis with respect to the effect of karyotype on the length of the larvae seemed to indicate that the Lonsdale, Barwon, and Corio inversions have at least some slight effect on larval length. The *Lo*, *Ba*, and *St^{Co}* sequences appeared to decrease the length of female larvae, but increase the length of male larvae. However, considering the difficulty of measuring larval length accurately and the small differences noted, the results available are not sufficiently convincing to merit detailed consideration.

TABLE 1

EFFECT OF KARYOTYPE ON VENTRAL TUBULE LENGTH IN *K. INTERTINCTUS*

Karyotypes are designated as follows: A, *St/St*; B, *St/Inv*; C, *Inv/Inv*. Values given are numbers of comparisons in which ventral tubule length in larvae of one karyotype were greater than, less than, or the same as that of another karyotype (for details of method of analysis see Section II). OG, Ocean Grove samples only. BG, Botanic Gardens samples only

Inversion and sample	A>B	A<B	A=B	P	B>C	B<C	B=C	P	A>C	A<C	A=C	P
Lonsdale inversion												
Females	40	69	16	0.01*	51	61	13	0.4	27	47	17	<0.05*
OG	18	32	8	0.09	31	39	9	0.4	15	32	9	<0.05*
BG	12	25	3	0.06	13	18	2	0.5	10	13	1	0.65
Males	30	41	14	0.3	32	35	8	0.85	26	26	12	1.0
Total	70	110	30	<0.01*	83	96	21	0.4	53	73	29	0.15
Barwon inversion												
Females	41	59	14	0.12	70	68	20	0.95	31	39	14	0.4
OG	16	34	4	0.02*	31	30	8	1.0	17	23	5	0.5
Males	27	43	5	0.08	41	37	17	0.8	24	30	6	0.55
BG	4	14	2	<0.05*	7	8	2	1.0	3	7	3	0.4
Total	68	102	19	<0.02*	111	105	37	0.75	55	69	20	0.3
Corio inversion												
Females	23	35	7	0.2	57	80	16	0.07	22	29	7	0.45
BG	6	4	2	0.35	12	28	5	0.03*	4	7	2	0.65
Males	46	40	13	0.65	10	14	9	0.65	5	7	4	0.8
Total	69	75	20	0.7	67	94	25	0.06	27	36	11	0.35

* Difference significant at the 0.05 level of probability.

The results of the analysis of the effect of karyotype on the length of the larval ventral tubules are given in Table 1. There are indications that the three inversions each affect ventral tubule length to some extent, and here the effect is similar in females and males.

For the Lonsdale inversion two comparisons were significant in female larvae. Thus *St^{Lo}/St^{Lo}* larvae had shorter ventral tubules than either *St^{Lo}/Lo* or *Lo/Lo*, the

heterozygotes possibly being intermediate in length. This can be seen in the mean lengths of the two largest collections from Ocean Grove in which this character was measured (Table 2). The differences, however, are not significant. The comparisons in males were not significant, but one comparison, St^{Lo}/St^{Lo} versus St^{Lo}/Lo , was again significant in the combined female+male data.

TABLE 2
MEAN VENTRAL TUBULE LENGTH OF FEMALE LARVAE OF *K. INTERTINCTUS* COLLECTED AT OCEAN GROVE, VICTORIA

Karyotype	No. of larvae	Mean length (mm)	S.D.	Karyotype	No. of larvae	Mean length (mm)	S.D.
Collected in January 1964				Collected in February 1964			
St^{Lo}/St^{Lo}	10	1.64	0.32	St^{Lo}/St^{Lo}	16	1.42	0.32
St^{Lo}/Lo	33	1.74	0.29	St^{Lo}/Lo	37	1.56	0.30
Lo/Lo	19	1.84	0.29	Lo/Lo	22	1.60	0.31
St^{Ba}/St^{Ba}	13	1.72	0.35	St^{Ba}/St^{Ba}	12	1.48	0.25
St^{Ba}/Ba	27	1.70	0.28	St^{Ba}/Ba	46	1.54	0.32
Ba/Ba	22	1.84	0.28	Ba/Ba	17	1.64	0.30

None of the comparisons involving all female or male larvae were significant with respect to the Barwon inversion. However, the comparison of St^{Ba}/St^{Ba} and St^{Ba}/Ba produced significant results in the females from the Ocean Grove samples, in the males from the Botanic Gardens samples, and in the combined results of females and males. In all cases the heterozygotes had longer ventral tubules. There is an indication that Ba/Ba larvae have longer ventral tubules than St^{Ba}/St^{Ba} larvae and this difference was significant in the earlier analysis (Martin 1966). The results for females from the two largest Ocean Grove samples do in fact indicate a greater mean length of the ventral tubules of Ba/Ba larvae than of those of St^{Ba}/St^{Ba} larvae (Table 2). In only one of the samples is the mean length in heterozygotes greater than that in St^{Ba}/St^{Ba} larvae. None of these differences in mean length were significant.

The only comparison involving the Corio inversion which gave a significant deviation from a 1:1 ratio was the comparison of heterozygous females against those homozygous for Co/Co in the Botanic Gardens samples. The total data and the data for females only are both approaching significance, suggesting that the Co/Co larvae may have longer ventral tubules. The same trend was apparent in the earlier analysis (Martin 1966), although not significant.

When the combined effects of the Lonsdale and Barwon inversions were investigated, the extremely small number of comparisons possible, ranging from 14 to 67, tends to hide any but the most extreme effects (Table 3). Although only one comparison is significant, there is a consistent pattern to the data which is shown also by the earlier analysis (Martin 1966). This pattern suggests that Lo/Lo , Ba/Ba have the longest ventral tubules, with St^{Lo}/Lo , St^{Ba}/Ba having intermediate and St^{Lo}/St^{Lo} , St^{Ba}/St^{Ba} having shorter ventral tubules. This is in agreement with what would be expected from the results of the inversions singly, but if Lo and Ba are taken

to be size-increasing while St^{Lo} and St^{Ba} are size-decreasing, it would be expected that the St^{Lo}/St^{Lo} , St^{Ba}/St^{Ba} karyotype would have the shortest ventral tubules of all. Instead it appears that St^{Lo}/St^{Lo} , Ba/Ba and Lo/Lo , St^{Ba}/St^{Ba} karyotypes, which each have one size-increasing and one size-decreasing sequence, have smaller ventral tubules.

TABLE 3

COMPARISON OF THE COMBINED EFFECT OF THE LONSDALE AND BARWON INVERSIONS ON THE LENGTH OF LARVAL VENTRAL TUBULES IN *K. INTERTINCTUS*

Comparison	A>B	A<B	A=B	P	Comparison	A>B	A<B	A=B	P
St^{Lo}/St^{Lo} , St^{Ba}/St^{Ba} (A) versus St^{Lo}/St^{Lo} , Ba/Ba (B)					St^{Lo}/St^{Lo} , St^{Ba}/St^{Ba} (A) versus Lo/Lo , St^{Ba}/St^{Ba} (B)				
Females	13	7	5	0.3	Females	7	3	0	0.3
Males	11	7	1	0.5	Males	2	2	0	—
Females + males	24	14	6	0.2	Females + males	9	5	0	0.4
St^{Lo}/St^{Lo} , St^{Ba}/St^{Ba} (A) versus St^{Lo}/Lo , St^{Ba}/Ba (B)					St^{Lo}/Lo , St^{Ba}/Ba (A) versus St^{Lo}/St^{Lo} , Ba/Ba (B)				
Females	15	15	3	1.0	Females	9	6	2	0.6
Males	8	10	1	0.8	Males	8	6	2	0.8
Females + males	23	25	4	0.9	Females + males	17	12	4	0.5
St^{Lo}/Lo , St^{Ba}/Ba (A) versus Lo/Lo , Ba/Ba (B)					St^{Lo}/Lo , St^{Ba}/Ba (A) versus Lo/Lo , St^{Ba}/St^{Ba} (B)				
Females	15	25	7	0.2	Females	8	4	1	0.4
Males	7	7	6	1.0	Males	3	4	1	—
Females + males	22	32	13	0.3	Females + males	11	8	2	0.65
St^{Lo}/St^{Lo} , Ba/Ba (A) versus Lo/Lo , Ba/Ba (B)					Lo/Lo , Ba/Ba (A) versus Lo/Lo , St^{Ba}/St^{Ba} (B)				
Females	4	12	4	0.1	Females	7	2	1	0.2
Males	1	9	1	0.04*	Males	2	3	0	—
Females + males	5	21	5	<0.01*	Females + males	9	5	1	0.5
St^{Lo}/St^{Lo} , St^{Ba}/St^{Ba} (A) versus Lo/Lo , Ba/Ba (B)					St^{Lo}/St^{Lo} , Ba/Ba (A) versus Lo/Lo , St^{Ba}/St^{Ba} (B)				
Females	10	9	1	1.0	Females	5	5	1	1.0
Males	3	10	3	0.15	Males	1	1	0	—
Females + males	13	19	4	0.35	Females + males	6	6	1	1.0

* Difference significant at the 0.05 level of probability.

IV. DISCUSSION

These results, despite the somewhat limited data, clearly indicate that the three inversions investigated have an effect on the morphology of the larvae. Although the two characters studied are both length measures, it is evident that the results observed are not due to a single effect since, as has been shown previously (Martin 1963), these characters are independent during the seasonal changes in larval size. Thus it is not unduly surprising that the inversions apparently have a different effect on larval and ventral tubule length.

In general the heterozygote is intermediate in size between the two homozygotes but sometimes it is the same size as one homozygote, or, in the case of the Lonsdale inversion and body length, it may even be larger than the other karyotypes. Presumably this simply reflects whether the genes concerned are additive in effect, show dominance, or are overdominant. It would, however, be unwise on the basis of these results to try to state categorically which sequences have each type of inheritance.

A certain significance can be attached to the effect of karyotype on ventral tubule length. It has previously been shown that *Lo* and *Ba* tend to be more common

in lower rainfall areas and therefore the basis of the non-random association between the Lonsdale and Barwon inversions may be that *Lo* and *Ba* enable larvae to withstand drying out or conditions of high water temperatures when pools are at low levels during summer (Martin 1965). Edward (1964) concluded that, in Western Australia at least, low oxygen levels due to high temperature and bacterial activity in the bottom deposits could well have a rigorous selective action on larvae of *K. intertinctus*. It has also been shown that the function of the ventral tubules appears to be in respiratory exchange (Harnisch 1954; Strenzke 1960) and that larvae living in oxygen-poor environments develop larger blood gills than those living in oxygen-rich environments (Haas 1957). In agreement with this it has been found that both *K. intertinctus* and the closely related *K. paratinctus* have longer blood gills as summer progresses, although the larvae themselves are smaller (Martin 1963, 1966). Unfortunately the data quoted in Table 2 for January and February 1964 do not appear to reflect this, but this is apparently because the January sample represents the hotter period. At the time the February sample was made, late in the month, conditions were much cooler.

The *Lo* and *Ba* sequences would therefore appear to give those larvae carrying them an advantage in conditions of low oxygen availability, such as occurs during times of high water temperature, by providing them with a greater area for respiratory exchange. An association favouring the occurrence of these sequences together would further increase this advantage if the effects were additive.

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