Polygenic Analyses of Morphological Characters in *Pieris rapae crucivora* BOISDUVAL (Lepidoptera: Pieridae).

I. Heritability Estimates¹

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Heritabilities of external morphologies (pupal weight, body weight, forewing length, median cell length, hindwing length) in *Pieris rapae crucivora* were estimated by quantitative genetic method (sib analysis). The experiment was done to know the type and force of natural selection acting on these traits that might be of ecological significance. The estimates were in the low to moderate range (0.2–0.9, 0.44 on average) for the characters. These results suggest that genetic variances were maintained in these characters of *Pieris rapae crucivora*.

INTRODUCTION

Most phenotypic characters of evolutionary interest (e.g., morphological, physiological and behavioral characters) are continuously varying traits (i.e., metric characters) (LANDE, 1982). Since these metric characters are controlled by polygenes, their genetic properties cannot be analyzed by the simple Mendelian method. However, some evolutionary biologists have succeeded in detecting phenotypic variations which have additive genetic attributes (ARNOLD, 1981 a, b; BOAG, 1983; DINGLE and HEGMANN, 1982), using quantitative genetic methods (FALCONER, 1981; ARNOLD, 1981 a). There are very few empirical studies, however, that have estimated quantitative genetic parameters, although evolutionary dynamic theories based on quantitative genetics have been developed in recent years (LANDE, 1979, 1982).

This paper reports the preliminary results of a series of polygenic analyses on morphological characters of ecological importance in *P. rapae crucivora*: pupal weight, body weight, forewing length, median cell length and hindwing length. Though this species has been well investigated by ecologists (YAMAMOTO, 1982; SUZUKI, 1978), its genetic properties are still largely unknown. OHSAKI (1979, 1980, 1982) reported niche differentiation among three closely related pierid butterflies (*P. rapae crucivora*, *P. napi* and *P. melete*) and clarified the essential features that might be related to the differentiation. Morphological differences are expected to have some relationship with the process of the niche differentiation during speciation in these three species. In the present paper the author analyzes genetic variances in morphological characters of *P. rapae crucivora* by quantitative genetic methods.

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MATERIALS AND METHODS

The experiment consisted of two replicates, and was undertaken between May–July, 1985. Genetic parameters were estimated by sib analysis. Eight sires and fifteen dams used in the analyses were collected as first instar larvae in the campus of Nagoya University twice in May and June 1985, and were reared under the condition of 25°C, 16 hr daylength until adult emergence. Adult males and females were marked individually, introduced into plastic cages under daylight, and allowed to copulate. Males copulated with from one to three females (see Fig. 1). Mated females were provided potted cabbage seedlings in clear plastic cages as oviposition sites. Most females laid eggs under the condition of 25°C, 1,500 lux. Progeny were transferred to clear plastic cups (9 cm in diameter at bottom) as soon as they hatched and were reared at

Fig. 1. An illustration for mating scheme in parent generation that allowed sib analysis. $M(i)$: i -th sire, $F(i, j)$: j -th dam in i -th sire.

Fig. 2. Schematic drawings describing measured wing dimensions of *P. rapae crucivora*.

20°C, 16 hr daylength, being supplied with fresh cabbage leaves (*Brassica oleracea*) every third or fourth day. Progeny derived from a single dam were apportioned into four rearing cups. When the progeny had reached the fourth instar they were sexed; four of each sex were chosen at random (using pseudo-random numbers) and transferred into single sex cups (four per cup). Pupae were removed from the rearing cups and fixed by binding agent in small plastic cups with enough space for adult emergence. Pupal weight was measured with a microbalancer (Chyo Jupiter 100) accurate to 0.1 mg as soon as pupae were hardened. Adult body weight was measured at 24 ± 1 hr after emergence with the above-mentioned balancer precise to 0.1 mg. Adults were then killed (thorax crushed), and wrapped in pieces of paraffin paper for storage at room temperature. After completion of all rearing experiments, adult samples were measured for dried body weight, forewing length, median cell length and hindwing length. Morphometrical definitions of wing dimensions are illustrated in Fig. 2. Wing shape was taken as the ratio of median cell length to forewing length and was transformed to Arcsin scale. Dried body weight was measured with the above-mentioned balancer precise to 0.01 mg. Forewing length, median cell length and hindwing length were measured with a slide caliper precise to 0.05 mm.

All available data were supplied for estimating variance components by hierarchical (nested) analysis of variance (ANOVA). Effects of sires, dams, rearing cups and residuals constructed four variance components, i.e., between-sire variance (V_s), between-dam variance (V_d), between-cup variance (V_c) and within-progeny variance (V_w), assuming a random effects model. Males varied in the numbers of mates and not all females could produce enough offspring to make four rearing cups. This, as well as experimental incompleteness, caused the data to be unbalanced. A calculation procedure for unbalanced data was therefore applied in this study (SOKAL and ROHLF, 1981). While ordinary F -values might be overestimates, SATTERTHWAITE's approximation gave adjusted F -values (SOKAL and ROHLF, 1981).

The variance components have numerical relationships with genetic and environmental variances as follows.

$$V_s = 1/4 V_A$$

$$V_d = 1/4 V_A + 1/4 V_D + (V_{EC})$$

V_A : additive genetic variance

V_D : dominance (non-additive genetic) variance

V_{EC} : common environmental variance (FALCONER, 1981)

While the between-sire variance is attributable only to genetic variance, the between-dam variance may generally involve common environmental variance. The common environmental variances were, however, excluded from the between-dam components by introducing between-cup (within-dam) variance components (V_c) in this study. Resemblance among individuals in each rearing cup within full sib families must have been caused by common environmental effects which presumably meant similar food quality and any other between-cup micro-environmental effects that could not be regulated experimentally.

Additive genetic variances were estimated by combined full-sib covariances using the following formula: $V_A = 2(V_s + V_d)$ (FALCONER, 1981), assuming dominance (non-additive genetic) variances were negligible. Heritability estimates are given as proportions of additive genetic variances to total phenotypic variances.

RESULTS AND DISCUSSION

A basic assumption in quantitative genetic analyses is that phenotypic values are, at least approximately, normally distributed. In order to test the adequacy of this assumption a χ^2 test for goodness of fit to the normal distribution was done for each character analyzed. The results of the tests for all characters are shown in Table 1.

Table 1. Normality tests (χ^2 tests) of phenotypic distributions

Trait	Sex	d.f.	χ^2
Pupal	m	8	7.89 ^{ns}
weight	f	6	4.75 ^{ns}
Body	m	5	7.72 ^{ns}
weight	f	4	2.02 ^{ns}
Dried	m	11	11.55 ^{ns}
body weight	f	8	5.70 ^{ns}
Forewing	m	6	11.39 ^{ns}
length	f	5	7.90 ^{ns}
Median cell	m	4	8.42 ^{ns}
length	f	2	3.38 ^{ns}
Hindwing	m	7	19.55 ^{**}
length	f	3	2.64 ^{ns}
Wing	m	5	1.58 ^{ns}
shape	f	3	2.49 ^{ns}

m: male. f: female. d.f.: degree of freedom.

^{ns} $P > 0.05$, ^{**} $P < 0.01$.

Table 2. Differences in phenotypic means between sexes. Significant levels were determined by *t*-statistics. When the variances were significantly different by *F*-tests, WELCHE's method was adopted (SNEDECOR and COCHRAN, 1967) to test differences of means

Trait	Sex	<i>N</i>	<i>M</i>	<i>S</i>	<i>CV</i>	<i>D</i>
Pupal	m	180	222.2	24.64	0.111	
weight	f	174	205.4	21.26	0.104	16.8***
Body	m	160	69.52	9.94	0.143	
weight	f	100	73.23	9.93	0.136	3.71**
Dried	m	155	26.24	3.93	0.150	
body weight	f	100	29.72	4.12	0.139	3.48***
Forewing	m	157	27.33	1.26	0.046	
length	f	115	26.78	0.96	0.036	0.55***
Median cell	m	158	14.31	0.74	0.052	
length	f	99	13.94	0.63	0.045	0.37***
Hindwing	m	153	23.57	1.32	0.056	
length	f	94	22.94	0.83	0.036	0.63***
Wing	m	154	46.35	0.91	0.020	
shape	f	96	46.05	0.72	0.016	0.30**

m: male, f: female, *N*: sample size, *M*: mean, *S*: standard deviation, *CV*: coefficient of variation, *D*: difference in mean.

** $P < 0.01$, *** $P < 0.001$.

Table 3. ANOVA tables of three-way nested analyses of variance for morphological characters in *Pieris rapae crucivora*. *F*-values, adjusted by SATTERTHWAITE's approximation, are shown in parentheses. *F*-tests were practiced separately using either the ordinary *F* values or adjusted ones

Pupal weight							
(Male)	<i>d.f.</i>	<i>m.s.</i>	<i>F</i>	(Female)	<i>d.f.</i>	<i>m.s.</i>	<i>F</i>
SIRE	7	955.43	0.34 ^{ns} (0.30 ^{ns})		7	1,131.71	1.31 ^{ns} (1.20 ^{ns})
DAM	7	2,826.57	3.66** (3.66**)		7	861.00	1.67 ^{ns} (1.67 ^{ns})
CUP	34	772.29	1.85**		36	515.42	1.37 ^{ns}
RES	128	417.18			123	375.17	
Body weight							
(Male)	<i>d.f.</i>	<i>m.s.</i>	<i>F</i>	(Female)	<i>d.f.</i>	<i>m.s.</i>	<i>F</i>
SIRE	7	436.49	1.41 ^{ns} (1.23 ^{ns})		7	417.51	1.63 ^{ns} (1.35 ^{ns})
DAM	7	308.69	2.12 ^{ns} (2.11 ^{ns})		5	255.66	1.99 ^{ns} (1.86 ^{ns})
CUP	33	145.52	2.49**		20	128.40	2.78**
RES	111	58.39			67	46.19	
Dried body weight							
(Male)	<i>d.f.</i>	<i>m.s.</i>	<i>F</i>	(Female)	<i>d.f.</i>	<i>m.s.</i>	<i>F</i>
SIRE	7	35.743	0.80 ^{ns} (0.66 ^{ns})		7	56.523	1.21 ^{ns} (0.97 ^{ns})
DAM	7	44.883	2.37* (2.36*)		5	46.664	2.25 ^{ns} (2.07 ^{ns})
CUP	33	18.955	1.70*		21	20.745	2.23**
RES	107	11.149			66	9.285	
Forewing length							
(Male)	<i>d.f.</i>	<i>m.s.</i>	<i>F</i>	(Female)	<i>d.f.</i>	<i>m.s.</i>	<i>F</i>
SIRE	7	2.053	0.40 ^{ns} (0.32 ^{ns})		7	1.874	0.81 ^{ns} (0.63 ^{ns})
DAM	7	5.166	3.21* (3.21*)		5	2.312	3.08* (3.10*)
CUP	33	1.609	1.23 ^{ns}		25	0.751	0.91 ^{ns}
RES	107	1.307			75	0.823	
Median cell length							
(Male)	<i>d.f.</i>	<i>m.s.</i>	<i>F</i>	(Female)	<i>d.f.</i>	<i>m.s.</i>	<i>F</i>
SIRE	7	1.671	0.92 ^{ns} (0.72 ^{ns})		7	2.039	2.78 ^{ns} (2.10 ^{ns})
DAM	7	1.820	4.34** (—)		5	0.734	2.28 ^{ns} (2.25 ^{ns})
CUP	33	0.419	0.99 ^{ns}		21	0.322	1.46 ^{ns}
RES	107	0.423			65	0.220	
Hindwing length							
(Male)	<i>d.f.</i>	<i>m.s.</i>	<i>F</i>	(Female)	<i>d.f.</i>	<i>m.s.</i>	<i>F</i>
SIRE	7	1.487	0.59 ^{ns} (0.48 ^{ns})		7	1.664	0.84 ^{ns} (0.62 ^{ns})
DAM	7	2.540	2.71* (—)		5	1.972	2.34 ^{ns} (2.27 ^{ns})
CUP	33	0.938	1.02 ^{ns}		21	0.842	1.98*
RES	105	0.915			60	0.425	

(Table 3 Continued)

Wing shape							
(Male)	<i>d.f.</i>	<i>m.s.</i>	<i>F</i>	(Female)	<i>d.f.</i>	<i>m.s.</i>	<i>F</i>
SIRE	7	7.004	3.44 ^{ns} (2.74 ^{ns})		7	2.884	9.74* (10.61 ^{ns})
DAM	7	2.036	3.21* (—)		5	0.296	0.86 ^{ns} (0.86 ^{ns})
CUP	33	0.635	1.64*		21	0.346	0.37 ^{ns}
RES	105	0.388			65	0.945	

d.f.: degree of freedom. *m.s.*: mean squares.

** $P < 0.01$, * $P < 0.05$, ^{ns} $P > 0.05$.

Table 4. *F*-values in significant tests of variances between full sib families using pooled sire+dam factors

Trait	Male	Female
Pupal weight	2.45*	1.93 ^{ns}
Body weight	2.56*	2.73*
Dried body weight	2.13*	2.53*
Forewing length	2.24*	2.74*
Median cell length	4.16**	4.64*
Hindwing length	2.15*	2.13 ^{ns}
Wing shape	7.11***	5.22***

F-values were calculated by following formula,

$$F = M(f)/M(c); \quad M(f) = (S(s) + S(d))/(D(s) + D(d)),$$

when $M(c)$: mean squares between cups, $S(s)$: sum of squares between sires, $S(d)$: sum of squares between dams, $D(s)$: degrees of freedom between sires, $D(d)$: degrees of freedom between dams.

^{ns} $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Deviations from normality were not significant at 5% level in every character except for male hindwing length.

Although this species does not show conspicuous sexual dimorphism, the differences in the morphometric means were all significant between sexes (Table 2). Thus, genetic analyses were done separately for each sex.

ANOVA tables are listed in Table 3 for all analyzed characters. Statistical significance is low in female characters when compared with male ones. This can be accounted for by the much smaller sample sizes in females. Since the degrees of freedom between sires and between dams were rather low, the between-sire and between-dam variances were pooled. The test of the null hypothesis, $kV_s + k'V_d = 0$ (k, k' : coefficients), was done against the between-cup and the residual variances. The results indicated significant variance between full sib families in all characters except for female pupal weight and female hindwing length (see Table 4). This suggests the existence of genetic variability in the measured morphological characters of this species.

Significant between-cup variances were detected for male pupal weight, male body weight, female body weight, male dried body weight, female dried body weight, female hindwing length and male wing shape, suggesting that phenotypic variances were attributable in part to common environmental effects which were presumably caused by

Table 5. Estimates of additive genetic variances and heritabilities in morphological characters

Trait	Sex	V_a	h^2
Pupal weight	m	172.8	0.29 ± 0.16
	f	84.4	0.16 ± 0.12
Body weight	m	43.2	0.40 ± 0.20
	f	56.6	0.55 ± 0.30
Dried body weight	m	3.95	0.25 ± 0.16
	f	7.79	0.45 ± 0.27
Forewing length	m	0.36	0.23 ± 0.15
	f	0.30	0.32 ± 0.21
Median cell length	m	0.26	0.47 ± 0.22
	f	0.32	0.77 ± 0.35
Hindwing length	m	0.20	0.20 ± 0.15
	f	0.24	0.34 ± 0.24
Wing shape	m	0.80	0.92 ± 0.31
	f	0.42	0.77 ± 0.36

The standard deviations were calculated by the approximation: $V(h^2) = 16 h^2 / N$ where N is total sample size. m: male, f: female, V_a : estimate of additive genetic variance, h^2 : heritability estimate.

variance in leaf quality or food resource limits among rearing cups. Therefore, it is suggested that the variance in leaf quality and food resource limits contributed to the between-cup variance based on environmental effects, and this decreased the testing power to detect genetic variance by increasing error variances, as well as by reducing the heritability estimates.

The experimental design was too complicated for the scale of this experiment. Since the degrees of freedom between sires and between dams (7 each) were low, relative magnitudes of the variances of the two groups could not be estimated efficiently, probably owing to large sampling variance and covariance between the two components ($COV(V_s, V_d)$) (OSBORNE and PATERSON, 1952). Therefore, the dominance variance could not be separated from the genetic variance, and exact derivation of narrow sense heritability was not feasible in this study. But under the assumption that the dominance variance, at least approximately, is negligible the combination of the two components based on full sib covariance may give the best estimate of additive genetic variance (FALCONER, 1981).

Estimates of additive genetic variances and heritabilities are shown in Table 5. Heritability estimates were low or modest ranging from 0.2 to 0.6 except for wing shape. Although these estimates are probably not precise and, furthermore, might be underestimated because of the small sample size and the large environmental effects mentioned earlier, this preliminary experiment showed that genetic variances have been maintained in morphological characters of *Pieris rapae crucivora*. But to obtain more precise estimates of additive genetic variance and heritability, further investigations with larger sample size under better rearing conditions are needed.

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