A STUDY OF WEIGHT VARIATION IN *APHIDIUS SMITHI* (HYMENOPTERA: APHIDIIDAE) A PARASITE OF THE PEA APHID, *ACYRTHOSIPHON PISUM* (HOMOPTERA: APHIDIDAE)

by

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A study of weight variation in Aphidius smithi (Hymenoptera: Aphidiidae), a parasite of the pea aphid, Acyrthosiphon pisum (Homoptera: Aphididae)

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ABSTRACT

A study of weight variation in Aphidius smithi (Hymenoptera:Aphidiidae), a parasite of the pea aphid, Acyrthosiphon pisum (Homoptera:Aphididae).

The pea aphid-A. smithi system was used as a model to investigate weight variation in an insect protelean parasite. Weight variation of individuals over time was examined by determining changes in individual wet weight after mummy formation and eclosion. The rate of wet weight loss is highest after mummy formation, at eclosion and at death; a minimum of 6.5% of the eclosion wet weight is lost in the first hour after eclosion. For comparing weight between individuals, weight variation within-individuals can be minimized by using pupa weight 9-days-after oviposition, vacated-mummy weight or adult dry weight as unbiased estimators of the relative weights of individuals.

The effect of host weight at oviposition on parasite weight was examined for hosts ranging in age from 0.5 days to 6.0 days (in 0.5-day increments).

Parasite weight increased as the weight (age) of the host at oviposition increased from 264µg (0.5 days) to 1464µg (4.0 days); further increases in host weight (age) at oviposition did not result in further increases in parasite weight. The possible causes and significance of the relationship between host weight at oviposition and parasite weight are discussed. The weight of male parasites was found to be significantly less than that of female parasites.

Geographic variation in weight existed between parasites sampled from field populations in Ashcroft and Kamloops, British Columbia. When reared under constant laboratory conditions, the weight of the F_1 parasite generations were statistically indistinguishable, a fact suggesting that the variation between the two populations was caused by extrinsic environmental differences between the two locations and not by intrinsic differences.

Artificial selection was used in an attempt to produce parasite populations having increased and decreased mummy weights. After 9 generations of selection, the two populations had diverged by 5.2%

(males) and 3.6% (females) of the mummy weight of the base population. Although examination over generations indicated significant divergence, differences between the two populations were significant only in 3 of the 9 male generations and 1 of the 9 female generations. Possible reasons for the low divergence in weight between the two populations are discussed.

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CHAPTER 1

GENERAL INTRODUCTION

Most traits, if not all, exhibit variation. It is this variation that makes the study of biology intriguing, complex and unpredictable. To study and understand a trait is to study and understand its variation. Variation between populations is essential for the formation of species. Variation between subpopulations or samples permits an evaluation of the effects of different chemical, physical and biological environments. Variation between individuals is essential for the study of genetics. Variation within individuals over time and space is an essential part of ecology and demography.

Most traits of ecological and evolutionary significance, such as fecundity, longevity, rate of development and size, exhibit continuous variation (Dobzhansky et al. 1977). Such 'ecological' traits have

a high level of variation within individuals over time, and are greatly affected by the environment. In many cases, the variation within individuals over time is so great, and of such fundamental significance, that ecologists will limit their study to this variation (e.g. life tables). Typological models are frequently constructed from such data (Chesson 1978).

The variation in 'ecological' traits within and between populations is well documented (Gould and Johnston 1972). Although the ecological and evolutionary significance of this variation is appreciated, there are few quantitative evaluations of its causes.

In order to understand 'ecological' traits, it is essential to increase our basic knowledge of the variation in these traits. In this thesis, weight variation was studied in Aphidius smithi Sharma & Subba Rao (Hymenoptera:Aphidiidae), a protelean parasite of the pea aphid, Acyrthosiphon pisum (Harris) (Homoptera:Aphididae). The objectives of the study were:

- To determine the individual weight variation over time in A. smithi, from pupation to death (and to use this information for the determination of the best sampling techniques

for individual adult weight);

- To examine the effect of host weight at oviposition on A. smithi adult weight;
- To examine A. smithi weight variation within and between field populations; and
- To determine the response to artificial selection for weight in A. smithi (and if the response is large, to use the populations so produced to study both correlated responses to selection and genotype-environment interaction).

In the above sequence of objectives, each objective provided information that allowed for a more thorough examination of the subsequent objective.

Weight was chosen as the trait to be studied for several reasons. The weight of an individual can easily and accurately be measured (with a gram electrobalance). Weight or size is an ecologically significant trait. It is a factor in predator-prey and host-parasite interactions (Hespenheide 1973; Holling 1964; Price 1972; Schoener 1971; Wilson 1975). It is correlated with many ecological parameters, such as courtship behavior (Ewing 1960) and fecundity and longevity (Murdie 1969; Takahashi 1956; Tantawy and Vetukhiv 1960; Woodroffe 1951). Clines in weight or size have been recorded in cricket frogs (Nevo 1973),

Drosophila robusta (Stalker and Carson 1947) and Drosophila subobscura (Misra and Reeve 1964). These clines suggest an adaption to environmental parameters. Geographic variation in body dimensions has been recorded in many organisms (Gould and Johnston 1972). Traits such as weight also have a potential for being used as indicators of population quality in mass rearing programs (Boller 1972) and in natural populations.

A. smithi was chosen as the organism used in this study for several reasons. The species is part of a host-parasite system that can easily be maintained in the laboratory. A large amount of research has been published on this organism. Aphid parasites are economically important, having been used extensively in the biological control of aphids.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 THE ORGANISM USED IN THE STUDY

Aphidius smithi Sharma & Subba Rao is a specific protelean parasite of the pea aphid, *Acyrthosiphon pisum* (Harris). During oviposition the female *A. smithi* deposits a single egg into the haemocoel of the host aphid. At 20°C, the embryonic stage is completed by day 3, after which the parasite larva continues to develop within the host haemocoel. The host stops growing on day 5, and the parasite begins active feeding on the host tissue on day 6 (Cloutier 1978). By day 8 the parasite has consumed all the internal host tissue and has spun a cocoon inside the cuticle of the eviscerated aphid. The eviscerated aphid is referred to as a mummy. The cocoon is attached by the grown larva to the plant surface along a small slit on the ventral surface of the mummy. Each mummy contains only one

developing parasite. Pupation occurs within the mummy, and at approximately day 14 the adult parasite emerges from the mummy.

As with other Hymenopteran insects, A. smithi has a haplodiploid genetic system. Males are haploid and develop from unfertilized eggs, and females are diploid and develop from fertilized eggs. Virgin females produce only male offspring, while mated females are capable of producing both male and female offspring.

2.2 LABORATORY COLONIES

Seeds of the broad bean, Vicia faba L. var. Exhibition Long-Pod, were obtained from Buckerfield's Ltd. and stored at 5°C to maintain their high germination. The plants produced from new seed shipments were checked before they were used in any experiment to ensure that the host plants were consistently high in quality. The seeds were planted in potting soil and the plants were grown in a greenhouse until they were 10-15cm in height, at which time they were used for the stock colonies and experiments.

A stock colony of virginoparous pea aphid, Acyrthosiphon pisum (Harris) was maintained on potted broad beans in a growth chamber at 18°C to 23°C under a diel cycle of 16L/8D of artificial light. Low population density and frequent plant changes resulted in minimal alate production. The original pea aphid source colony was collected in 1972 on alfalfa, Medicago sativa L, near Kamloops, British Columbia.

A portion of the pea aphid stock colony was used as hosts for the A. smithi stock colony, which was kept in the laboratory at room temperature under a diel cycle of 16L/8D of artificial light. Low aphid densities and pre-mummy plant changes produced a high level of parasitism and large healthy parasites. The

A. smithi stock colony was originally collected in 1969, and was supplemented with more field collected parasites in the summer of 1976. Both A. smithi collections were made near Kamloops, British Columbia, in the same fields from which the pea aphid stock colony was collected.

2.3 EXPERIMENTAL TECHNIQUES

2.3.1 Host aphids for oviposition

Young adult aphids were taken from the stock colony and placed on cut plants in small plastic cages similar to those described in Mackauer and Bisdee (1965). The adults were allowed to produce offspring for 5 hours, after which they were removed from the plants. The cages containing the nymphs (approximately 20 per cage) were then transferred to a growth chamber at 20±1°C and constant light, where they remained until they reached the desired age for an experiment. All cages in the growth chamber were rotated every day to compensate for minor differences in temperature between locations within the growth chamber.

2.3.2 Parasites for oviposition

Mummies were removed from the plants in the parasite stock colony 48 hours before the beginning of oviposition and individually placed into size 00 gelatin capsules. Those parasites that had emerged by 12 hours before the beginning of oviposition were set aside as potential parasites for mating. The parasites were paired up and permitted to mate inside the gelatin capsules. The time of mating varied between experiments. Each male was allowed to mate only once. Only those females that were observed in copula were

used for oviposition.

2.3.3 Oviposition

Each ovipositional session was approximately one hour in duration. Ten minutes before the beginning of the ovipositional session the host aphids were removed from the plants in the cages (4-9 cages per session, depending upon the experiment), and placed into small petri dishes (one petri dish per cage). As a control, 2 to 5 host aphids per petri dish were randomly selected and placed into an oven for drying. Each ovipositional session was subdivided into 3 approximately 20-minute subsessions or 2 approximately 30-minute subsessions (depending upon the experiment). During each subsession, each of 7 to 10 parasites were used to oviposit into 2 to 5 host aphids (each from a different petri dish). The same parasites were used in each subsession of a session. The oviposition was carefully observed inside a gelatin capsule to ensure that each host aphid was attacked only once. At the end of the ovipositional session the potentially parasitized aphids were placed (randomly or by parent, depending upon the experiment) on plants in 6 to 10 small plastic cages (15-20 aphids per cage). The cages containing the parasitized aphids were then transferred to a growth chamber at 20±1°C and constant light. All cages in the

growth chamber were rotated every day. The host plants were changed every 2 to 3 days.

The age of the host aphids at oviposition was taken from the midpoint of the ovipositional session. Thus, 48-hour old host aphids were 48±2.5-hours old at the midpoint of the ovipositional session. Since the ovipositional session was 1 hour in duration, these aphids would have been 48±3 hours old at the time of oviposition.

2.3.4 Mummy removal, eclosion, drying and weighing

At 7.5 to 10 days after oviposition (depending upon the experiment) the mummies containing the develcping parasites were removed from the plant surface, placed into numbered size 00 gelatin capsules and returned to the growth chamber. Eclosion occurred within the gelatin capsules. The emerging parasites were exposed to constant light in the growth chamber, since A. smithi does not normally emerge in the dark phase of the diel cycle (Mackauer and Henkelman 1975), and the effect of delayed eclosion upon weight is not known. The emerged parasites were kept inside the gelatin capsules until after death.

The parasites and the control aphids were oven dried at 100°C until desiccation terminated. The empty mummies were not oven dried since oven drying did not

reduce their weight.

The weights of the individual control aphids, parasites and mummies were determined on a Cahn model G-2 Electrobalance set at 1mg full range. Tare weights were used for weighings over 1mg. Live parasites were anaesthesized with CO_2 before being weighed.

CHAPTER 3

INDIVIDUAL PARASITE WEIGHT : VARIATION OVER TIME AND SAMPLING TECHNIQUES

3.1 INTRODUCTION

At any given time in a natural population, much of the observed variation in most 'ecological' traits is caused by variation within individuals over time. The size, fecundity, behavior and energy requirements of an individual are all, in part, dependent upon the age of the individual. Much of ecology has been concerned with the study of this variation within-individuals.

A. smithi develops from a minute egg into an adult parasite in 178.6 degree days (Campbell and Mackauer 1975a). A developing A. smithi continually increases in weight from the egg stage until the larva has consumed its entire host and the mummy is formed. The dry weight of the parasite decreases 20-30% between mummy formation and eclosion (Cloutier 1978). After eclosion,

the weight of an adult insect will be influenced by a multitude of factors such as temperature, relative humidity, physical activity and feeding (Wigglesworth 1972). Little is known about post-eclosion weight changes in A. smithi.

Weight variation within an individual can be a serious source of error when studying weight variation between individuals. For such studies, it is essential to choose a sampling technique for individual weight that will minimize the within-individual component of weight variation. There are several possible approaches to the sampling of individual weights in a population of A. smithi. The weights could be measured at some physiologically defined point during the development of each individual. The whole population could be weighed at some specific time after oviposition. Relative live adult weights could be estimated by measuring some other highly correlated variable. Choosing between the above approaches requires knowledge of how weight varies in an individual A. smithi over time.

In this chapter the relationship between parasite wet weight and time after oviposition is examined from mummy formation to desiccation after death. Also, the rate of wet weight loss after eclosion is examined. Knowledge of these relationships is used

to assess the various techniques for sampling individual adult weights. Specific techniques are selected for use in subsequent experiments.

3.2 MATERIALS AND METHODS

Four plastic cages were used to rear 48±2.5 hour old host aphids. Five 'control' aphids were sampled from each cage prior to oviposition. One hundred potentially parasitized aphids were obtained by allowing each of 10 mated female parasites to oviposit into 5 host aphids approximately every 30 minutes for approximately 1 hour. The parasites were mated 6 hours before the beginning of the ovipositional session. The potentially parasitized aphids were placed on plants in 10 plastic rearing cages (10 aphids per cage), one cage for all the aphids attacked by one female parasite, and allowed to develop. The mummies containing the developing parasites were removed from the plant surface 7.5 days after oviposition (approximately 0.5 days after mummy formation).

The relationship between parasite wet weight and time after oviposition was determined by weighing each mummy (including the developing parasite it contains) every 8 hours from 192 hours (8 days) after oviposition to the beginning of eclosion, 272 hours (11.3 days) after oviposition. The parasites were not weighed collectively at specific times after oviposition during the eclosion period. After eclosion was complete each parasite was weighed every 12 hours from 296 hours

(12.3 days) after oviposition, through death, until 404 hours (16.8 days) after oviposition; then every 24 hours until 524 hours (21.8 days) after oviposition. The parasites were kept inside the gelatin capsules throughout the experiment, except when they were being weighed. The sequence of individual weighings for each of the above weighing sessions was kept constant to reduce error, since each weighing session required 90 minutes to complete. The weight of a developing parasite in the pre-eclosion section of the curve was calculated by subtracting the weight of its mummy (as determined below) from the weight of the pupa-mummy complex. Average wet weight losses were calculated between each of the sampling times.

The wet weight loss after eclosion was determined by weighing each parasite within 15 minutes after eclosion, and again 1, 2, 4 and 8 hours after eclosion. The average and cumulative weight losses for the first 8 hours after eclosion were calculated from the data. Eclosion times were recorded.

Room-dried adult weight, oven-dried adult weight, and mummy weight were selected as potential variates for estimating the live adult weight of the parasite. Each parasite was weighed after being room-dried until the weight stabilized, and again after being oven-dried

until the weight stabilized. Each mummy was weighed 2 hours after eclosion. The stability of the mummy weights was checked by weighing a subsample of the mummies at 25 days after oviposition (approximately 13 days after eclosion).

Correlation coefficients between the values obtained using the various sampling techniques were calculated by computer.

3.3 RESULTS AND DISCUSSION

3.3.1 Variation in wet weight within individual parasites

The curves in Fig. 1 show the decrease in mean wet weight of the parasites after mummy formation as a function of the time after oviposition. The eclosion sections of the curves were estimated by interpolation. Descriptive statistics on these data are presented in Table 1. Eclosion occurred over a 16 hour period in both sexes, with eclosion in the females beginning 4 hours later than eclosion in the males. The mean (±S.E.) weight of the control aphids was 60.15 ± 1.38µg.

The shape of the curve is similar for the two sexes, with the female weights being consistently greater than the male weights. The pre-eclosion weights show high rates of weight loss between 192 and 208 hours, and again between 248 hours and the beginning of eclosion. The high rate of weight loss at these times is probably due to the high rate of metabolic activity required for the two metamorphic moults. The population continues to have a high rate of weight loss between the eclosion of the first male and the last female. Values for portions of this period cannot be calculated from the data since the population was not weighed collectively during this time. The rate of weight loss is constant during the adult life inside

Figure 1. Decreases in wet weight after mummy formation in male and female Aphidius smithi.

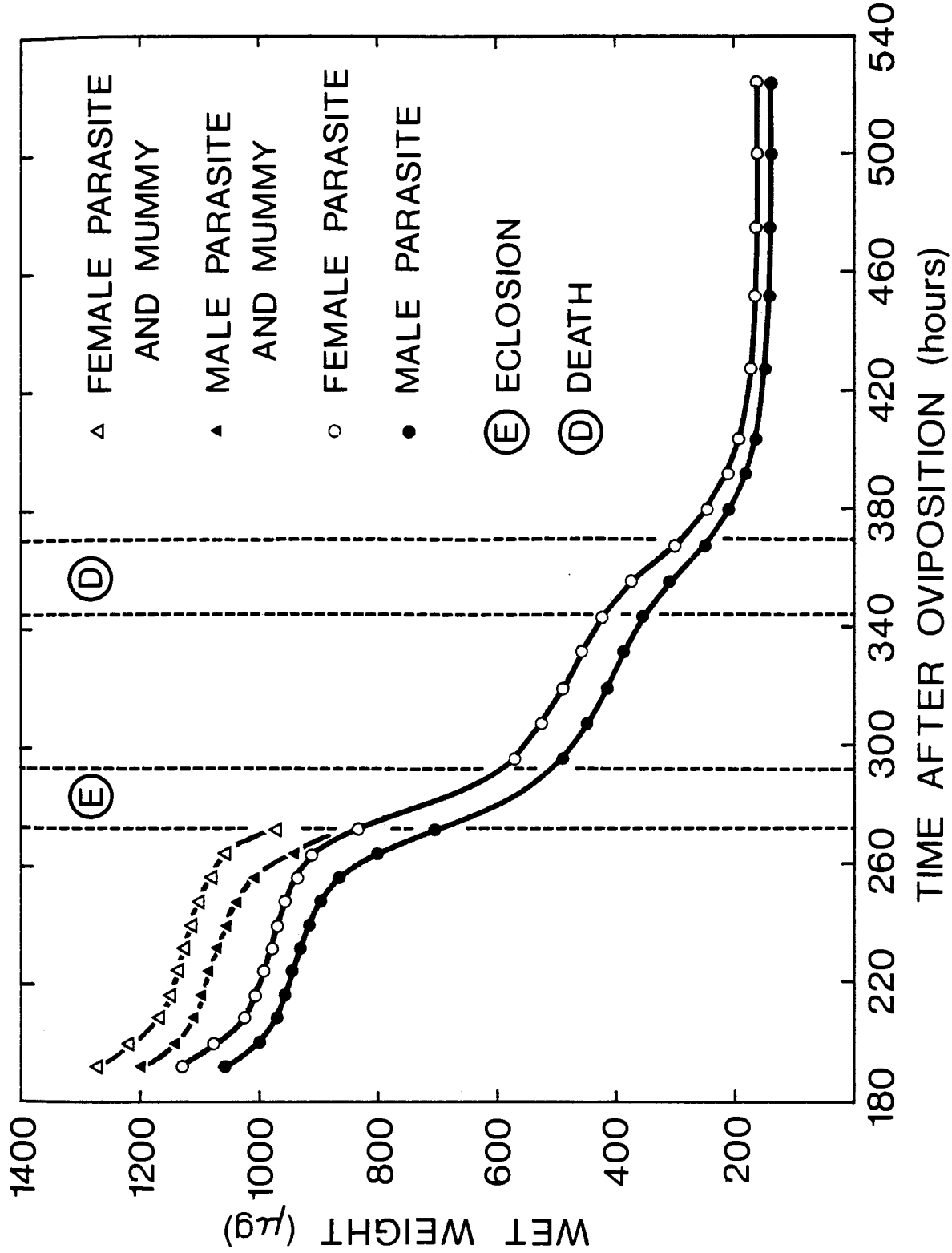

FEMALE PARASITE AND MUMMY
MALE PARASITE AND MUMMY
FEMALE PARASITE
MALE PARASITE
E ECLOSION
D DEATH
WET WEIGHT (μg)
1400
1200
1000
800
600
400
200
180
220
260
300
340
380
420
460
500
540
TIME AFTER OVIPOSITION (hours)

Table 1 Descriptive statistics on wet weight losses after mummy formation in (A) male and (B) female Aphidius smithi.

A) Hours after oviposition	N	Mean wet weight (µg) a)	Standard error mean	C.V.	Average weight loss (µg/hr)
192	61	1200	14	9.20	
200	61	1139	13	8.95	7.6
208	61	1107	13	8.89	4.0
216	61	1095	12	8.80	1.2
224	61	1082	12	8.85	1.6
232	61	1070	12	8.79	1.2
240	61	1053	12	8.81	2.1
248	61	1037	12	8.82	2.0
256	61	1003	11	8.83	4.2
264	61	940	12	9.82	7.8
272	61	844	11	10.42	12.0
296	61	488	6	9.99	9.8
308	61	448	6	10.42	3.3
320	61	413	6	10.68	2.9
332	61	385	5	11.05	2.3
344	61	354	6	14.31	2.6
356	61	307	9	22.04	3.9
368	61	248	9	28.71	4.9
380	61	209	8	31.09	3.2
392	61	179	7	30.57	2.5
404	61	162	6	28.53	1.4
428	61	151	4	20.47	0.5
452	61	144	2	13.01	0.3
476	61	139	2	8.64	0.2
500	61	138	2	8.64	0.1
524	61	138	2	8.72	

a) includes the weight of the mummy from 192 to 272 hours after oviposition

Table 1 Continued

B) Hours after oviposition	N	Mean wet weight (µg) [a]	Standard error mean	C.V.	Average weight loss (µg/hr)
192	14	1270	35	10.21	
200	14	1217	33	10.29	6.6
208	14	1167	29	9.29	6.2
216	14	1147	28	9.15	2.5
224	14	1134	28	9.14	1.6
232	14	1122	28	9.26	1.5
240	14	1112	27	9.20	1.2
248	14	1098	27	9.33	1.7
256	14	1077	28	9.65	2.6
264	14	1053	27	9.74	3.0
272	14	970	32	12.36	10.4
296	14	571	17	11.31	10.6
308	14	525	16	11.46	3.8
320	14	488	15	11.80	3.1
332	14	455	15	12.45	2.7
344	14	423	16	14.37	2.7
356	14	371	22	21.70	4.2
368	14	298	26	32.68	6.1
380	14	243	25	39.01	4.6
392	14	209	24	42.46	2.8
404	14	191	19	37.86	1.5
428	14	170	12	27.45	0.9
452	14	164	9	21.23	0.3
476	14	159	6	14.27	0.2
500	14	157	5	11.66	0.1
524	14	155	4	10.21	0.1

a) includes the weight of the mummy from 192 to 272 hours after oviposition

the gelatin capsule, increasing slightly during death. It is interesting to note that the rates of weight loss in the early and late pupal stages are higher than those of an active (inside a gelatin capsule) adult parasite. The dead parasites have dried to constant weight by about 20 days after oviposition.

Weight losses at specific points during the development of an individual, such as moulting, eclosion and death, would be much more dramatic than those illustrated in Fig. 1. The high population variation in developmental rates (20 hour range at eclosion), combined with the length of time between weighings, resulted in the points of individual high weight loss being scattered over a wide interval. This is illustrated by the high rate of weight loss at eclosion, at which time the physiological ages of the parasites are synchronized (Table 2). Both sexes lose at least 6.5% of their eclosion wet weight during the first hour after eclosion. The population wet weight loss during the 20 hour eclosion period averaged only 1.5% of the eclosion wet weight per hour when all individuals in the population were sampled at the same time after oviposition. This sampling technique failed to reveal the dramatic 6.5% weight loss during the first hour after eclosion. Katz and Young (1975) found

Table 2 Wet weight losses after eclosion in (A) male and (B) female Aphidius smithi.

A)

Hours after eclosion	N	Mean wet weight (µg)	Standard error mean	C.V.	Average weight loss (µg/hr)	Cumulative percentage weight loss a)
0	60	625	8	10.23		
1	60	583	7	9.30	42	6.7%
2	60	571	7	9.43	12	8.6%
4	60	552	7	9.42	10	10.3%
8	60	527	6	9.54	6	11.2%

B)

Hours after eclosion	N	Mean wet weight (µg)	Standard error mean	C.V.	Average weight loss (µg/hr)	Cumulative percentage weight loss a)
0	14	679	17	9.30		
1	14	628	15	8.97	48	7.1%
2	14	611	15	8.99	17	9.6%
4	13	597	16	9.77	7	10.6%
8	10	568	18	10.29	7	11.6%

a) calculated as a percentage of the eclosion wet weight

similar high wet weight fluctuations at emergence in Drosophila melanogaster. The initial high rate of weight loss is probably due to evaporation when the adult insect is first exposed to the air.

Variation in wet and dry weight within an individual adult insect is highly dependent upon the environment in which the insect lives. Variation in water content will be dependent upon the rates of water evaporation, defecation, ingestion and absorption (Burcell 1974), which in turn are dependent upon many environmental factors. Variation in dry weight will be dependent upon feeding rates and the efficiency of food utilization, which are again dependent upon many environmental factors. Thus the data presented in Fig. 1 and Table 1 are only valid for the environment in which the experiment was performed. Although this is only one of a myriad of environments in which A. smithi could exist, the data do illustrate the relative rates of weight loss at the various stages after mummy formation.

3.3.2 Standardizing the adult weight of an individual

In studies on weight variation between individuals, it is essential that the weight of each individual be standardized such that the within-individual component of weight variation in the population is

minimized. The need for such a standardization is apparent from the magnitude of the mean within-individual weight variation observed in Fig. 1. There are several approaches to the standardization of individual weights.

One approach is to sample each individual at some point during its development that is both easy to observe and short in duration. By using this approach, the within-individual component of weight variation in the population should approach zero. The only suitable point during the development of A. smithi is eclosion.

One disadvantage to sampling weight at eclosion is that sampling must be carried out over the entire eclosion period for the population. The length of the eclosion period can be reduced by keeping all the relevant environmental parameters as constant as possible. Temperature and relative humidity are known to affect developmental rates in A. smithi (Wiackowski 1962). In the tightly controlled environment in which the experiment described in this chapter was run, the eclosion period was still 20 hours. It is technically unrealistic to sample weights over a 20 hour period for each of a series of experiments.

Another disadvantage to sampling weight at eclosion is that errors will be introduced due to the

high rate of weight loss during the first hour after eclosion (Table 2). In this experiment, with emergence checks every 10 minutes, some parasites were not weighed until 15 minutes after they emerged from the mummy. The presence of error introduced by such delays is suggested by the correlation coefficients between the values obtained at various times after eclosion in the males (Table 3). Wet weights 1, 2, 4 and 8 hours after eclosion are highly correlated with each other but are not as highly correlated with wet weight at eclosion. Due to the high rate of weight loss at eclosion, wet weight one hour after eclosion is probably a more accurate estimate of relative individual adult weight.

A second approach to the sampling of individual adult weight is to sample the whole population at a particular length of time after oviposition. Sampling at any time after mummy formation may be indicative of adult weight since the parasite completes its feeding before mummy formation. The advantage of this approach is that it is technically convenient. With short parasitization sessions, all the individuals from each session can be weighed at the same sitting. One disadvantage of this approach is that it cannot be used on natural populations since the time of oviposition is

Table 3 Correlation coefficients between wet weights at specific times after eclosion in male *Aphidius smithi* (N=60).

first variate	second variate T1	T2	T3	T4
eclosion (=T1)	1.000			
1 hour after eclosion (=T2)	0.941	1.000		
2 hours after eclosion (=T3)	0.935	0.991	1.000	
4 hours after eclosion (=T4)	0.918	0.992	0.983	1.000
8 hours after eclosion (=T5)	0.903	0.980	0.972	0.995

unknown in field situations. Another disadvantage is that even when applied to synchronous laboratory populations, each individual will be at a different stage of development at any given length of time after oviposition due to differences in developmental rates between the individuals. The magnitude of the error introduced by these different developmental rates can be minimized by sampling at a time when the mean rate of weight loss for the population is low. Sampling at such a time will also reduce the error when comparing the results of different experiments which may have been run under slightly different environmental conditions. The rate of weight loss in A. smithi is minimal between 208 and 248 hours after oviposition (Fig. 1, Table 1). Thus, using this approach, the optimal time for sampling the individual adult weights in A. smithi (under the environmental conditions of this experiment) would be at any specific time between 208 and 248 hours after oviposition.

A third approach to the sampling of individual adult weights is to sample a trait that is constant over time and indicative of the live adult weight. Two such traits that exist in A. smithi are weight of the vacated mummy and dry weight of the parasite after death. There are several advantages to using this

approach. It is technically very convenient since the weights are constant over time and therefore can be determined at the convenience of the experimenter. There is no error introduced by differences in physiological age between individuals since the traits represent a constant physiological state of the individual. The disadvantage of this approach is that since these traits are not perfectly correlated with the live adult weight, error will be introduced when using them as indicators of live adult weight.

Data on weight of the mummy and dry weight of the parasite after death are presented in Table 4. All the variables are highly correlated with wet weight one hour after eclosion. The values at which room dried weights stabilize will be dependent upon the environmental conditions in the room. Thus, oven dried weight is a better variable since the environment is described in more detail. This technique for measuring weight has limited use since the dead parasites cannot be be used as parents for subsequent generations.

Weight of the mummy is an interesting variable. In this experiment the mummy weight was found to be constant from 3 hours after eclosion to 13 days after eclosion. The mummy includes the cuticle of the eviscerated aphid, the cocoon and the meconium. The

Table 4 Descriptive statistics on mummy weight, room dried parasite weight and oven dried parasite weight in male and female *Aphidius smithi*.

Variable	N	Mean weight (µg)	Standard error mean	C.V.	Correlation coefficient a)
vacated male mummy weight	60	140	2	9.90	0.897
room dried male parasite weight	60	133	1	8.60	0.873
oven dried male parasite weight	60	121	1	8.29	0.890
vacated female mummy weight	14	143	4	9.47	0.900
room dried female parasite weight	14	150	4	10.67	0.925
oven dried female parasite weight	14	136	4	9.66	0.900

a) correlation with wet weight 1 hour after eclosion

weight of the cocoon and meconium should be indicative of the weight of the developing parasite since the cocoon must surround the parasite and the meconium is a product of parasite metabolism. Although the weight of the eviscerated aphid will be dependent upon initial host size, it is also dependent upon the host-parasite interaction. For example, this interaction could result in delayed host killing, which would result in an increase in weight in both the eviscerated aphid and the adult parasite. Weighing mummies is a convenient technique for estimating individual adult weights that is independent of differences in physiological ages.

The preceding discussion has revealed that there are several possible techniques for sampling individual adult weight in A. smithi. Of the available techniques, weight of the vacated mummy, weight of the pupa (including the mummy surrounding it) at 9 days after oviposition, and weight of the oven dried adult will be used in this study since they are considered to be technically feasible and representative of adult weight. The particular technique from these three used in each of the subsequent experiments has depended upon the feasibility of each technique in each experimental situation.

When determining the effect of host size on

parasite weight (Chapter 4) all three techniques were used to estimate individual adult weights. This allowed for a determination of how host size affected each of the three variables independently. When sampling the distributions of adult weights in the field (Chapter 5), the weight of the oven dried adult was used to estimate adult weight. Pupal weight 9 days after oviposition could not be used since the time of oviposition was not known. Weight of the mummy was not used since mummies collected in the field tend to be very damaged (i.e. aphid appendages missing). When artificially selecting for heavy and light populations (Chapter 6), the weight of the mummy was used to estimate adult weight. Weight of the oven dried adult could not be used since live parasites were needed to parent subsequent generations. Weight of the pupa at 9 days after oviposition was not used since minor differences in the environments between the generations during the 4 month experiment would have affected the physiological age, and thus the weight, at 9 days after oviposition. Mummy weight is also a more convenient variable to measure than pupal weight at 9 days after oviposition.

3.4 SUMMARY

The wet weight of an adult A. smithi continuously decreases from mummy formation until after death when it is kept inside a gelatin capsule. The rate of wet weight loss is highest just after mummy formation, at eclosion and at death. The parasites lose at least 6.5% of their eclosion wet weight in the first hour after eclosion.

In studies on weight variation between individuals, the sampling technique used to determine the weight of each individual must be carefully chosen in order to minimize the within-individual component of the weight variation. Although weighing each individual one hour after eclosion would provide accurate data, it is technically not feasible. Weighing the vacated mummy, weighing the pupa (including the mummy surrounding it) at 9 days after oviposition, and weighing the oven dried adult are technically feasible and reasonably accurate techniques for determining the relative adult weights of the individuals in a population.

CHAPTER 4

THE EFFECT OF HOST WEIGHT AT OVIPOSITION ON PARASITE WEIGHT

4.1 INTRODUCTION

In the preceding chapter, it was demonstrated that the difference in physiological age between individuals in a population is one of the causes of population weight variation. Another possible cause of population weight variation is variation in the age or size of the host insect. Jackson (1937) found that in Pimpla turionellae, a pupa parasite of many species of Lepidoptera, the size of an individual was related to the size of its host. Salt (1940) demonstrated that in Trichogramma evanescens, an egg parasitoid of many insect species, the size of an individual is largely dependent upon the size of the egg in which it developed. Salt also used different sized eggs from one species as hosts, and again found a positive

correlation between the size of an individual and the size of its host.

In the pea aphid-A. smithi host parasite system, the size of the host is determined primarily by the physiological age of the host. Adult virginoparous of the pea aphid are viviparous. Development consists of simple metamorphosis (four nymphal instars) with adults being produced after 7-8 days at 20°C. A. smithi will oviposit into, and is capable of completing its development in, pea aphids ranging in age from birth to at least 21-days old (at 20°C) (Campbell and Mackauer 1975b). The total amount of food available for the developing parasite is not present at oviposition, as it was with T. evanescens and P. turionellae, since the parasitized aphid continues to feed for at least 5 or 6 days after being parasitized (Cloutier 1978). However, the amount of food available for the developing parasite larva will still be determined, to a large extent, by the physiological age (or size) of the host aphid at oviposition.

In this chapter, the effect of pea aphid weight at oviposition on A. smithi weight is examined. Host weight is varied by varying the age of the host aphids. The experimental design also permits an analysis of weight differences between males and

females. Replication factors are used to determine the reproducibility of the results. Parasite weights are determined by measuring pupa weight, mummy weight and adult weight. Measurement of all three variates demonstrates how host size affects each of the three variates individually.

4.2 MATERIALS AND METHODS

Four plastic cages were used to rear each of 12 ages of host aphids ranging in age from 12±2.5 hours to 144±2.5 hours in increments of 12 hours. For each age of host aphid, 56 to 80 potentially parasitized aphids were obtained by allowing each of 7 mated female parasites to oviposit into 2 to 4 host aphids approximately every 20 minutes for approximately 1 hour. Different female parasites were used for each age of host aphid. The parasites were mated 5 to 9 hours before the beginning of the ovipositional session. A random sample of 5 host aphids was removed at the beginning of each ovipositional session and oven-dried for subsequent determination of the weight of the host aphids at oviposition. The potentially parasitized aphids from each ovipositional session were randomly placed on plants in 4 plastic cages (15 to 20 aphids per cage) and allowed to develop. The mummies containing the developing parasites were removed from the plant surface 8 days after oviposition (approximately 1 day after mummy formation).

The individual pupa weights were determined by weighing each developing parasite (including the mummy surrounding it) 9 days after oviposition. The individual mummy weights were determined by weighing

each vacated mummy 2 to 4 days after eclosion. The weights of mummies frequently needed to be adjusted for missing aphid appendages or eclosion lid. The relationships between total mummy weight and mummy part weights, used for calculating the adjustment factors, were determined by weighing 5 antennae, 5 hind legs, 5 front legs and 5 emergence lids from mummies having each of the following weights (±1μg): 90μg, 120μg, 160μg, 180μg, 200μg, and 240μg. The individual oven dried adult parasite weights were determined by weighing each parasite approximately 14 days after eclosion (after 4-5 days of oven drying).

The entire experiment was repeated except that in the second run the oven dried parasite weights were not determined (and the mummy-part adjustment factors were not recalculated).

The parasite weight variance for each of the three approaches to measuring parasite weight (i.e. measuring pupa weight, mummy weight and adult weight) was analyzed separately.

An understanding of the experimental design is essential for an understanding of the approach taken to the analysis of variance. With pupa weight and mummy weight, each of the 1 to 13 parasites, from each sex, from each of 4 cages, from each of 12 ages of host

aphid, and from each of 2 experiments was weighed. Thus, four main factors are involved: sex, cage number, host age and experiment number. Cage number is nested under host age and experiment number. All of the other main factors are fully crossed. Experiment number and cage number are replication factors (Winer 1971). The 1 to 13 individuals of one sex within each cage provide the error term for the variance analysis. With adult weight, the design is identical except that the experiment number replication factor is not present since the adults were not weighed in the second experiment.

There are many reasons for using replication factors in this experiment. Replication factors were the only way of increasing the sample size for each host age, since the number of parasites that could be reared in a single plastic cage was limited. Including cage number as a replication factor in the variance analysis provided a means of checking for the presence of, and if present, eliminating, the variance component caused by different rearing conditions between cages. Including experiment number as a replication factor in the variance analysis provided a means of checking for the presence of, and if present, eliminating, the variance

component caused by running the experiment at different chronological times. It provided an indication of the ability to replicate the entire experiment.

The replication factors are random factors since the experimenter has no control over factors which may make Experiment A different from Experiment B or Cage 1 different from Cage 4, nor is he able to duplicate the conditions in the various experiments or cages. Sex is a fixed factor since the sex of an individual is known with certainty. Host age was analyzed as a fixed factor since the ages of the hosts were selected by the experimenter and are reproducible.

Taking all of the above information on the factors into consideration, the structural model used for the analysis of variance in pupa weight and mummy weight is:

$$\overline{ABCD}_{ijkl} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \gamma_k + \alpha\gamma_{ik} + \beta\gamma_{jk} + \alpha\beta\gamma_{ijk} + \delta_{l(jk)} + \alpha\delta_{il(jk)} + \bar{\varepsilon}_{ijkl}$$

where:

$\overline{ABCD}_{ijkl}$ = the mean observation in cell ijkl

μ = the parametric grand mean

α_i = the fixed effect of sex (factor A)

β_j = the fixed effect of host age (factor B)

$\alpha\beta_{ij}$ = the effect of sex-host age interaction

γ_k = the random effect of experiment number (factor C)

$\alpha\gamma_{ik}$ = the effect of sex-experiment number

interaction

βY_{jk} = the effect of host age-experiment number interaction

$\alpha\beta Y_{ijk}$ = the effect of sex-host age-experiment number interaction

$\delta_{l(jk)}$ = the random effect of cage number (factor D) (nested)

$\alpha\delta_{il(jk)}$ = the effect of sex-cage number interaction (nested)

$\bar{\varepsilon}_{ijkl}$ = the mean experimental error in cell ijkl

Since the adult weight measurements do not have an experiment number replication factor, the structural model used for the analysis of variance in adult weight is:

$$\overline{ABD}_{ijl} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \delta_{l(j)} + \alpha\delta_{il(j)} + \bar{\varepsilon}_{ijl}$$

A program titled 'Analysis of Variance and Covariance Program', available at the Simon Fraser University Computing Centre, was utilized for the variance analyses. An ANOVA with regression computer program was written for determining the relationship between host weight at oviposition and parasite weight. A computer program was written for determining the correlations between each of the weight variates at each host age.

4.3 RESULTS AND DISCUSSION

4.3.1 Adjustment factors for incomplete mummies

The relationships between the weight of a mummy and the weight of its aphid appendages and eclosion lid are presented in Fig. 2. Careful handling of the mummy, especially when removing it from the plant surface, resulted in all aphid appendages remaining attached to the mummy prior to eclosion. Thus, there was no need to adjust the pupa weights for missing mummy parts. The movement of the adult parasite inside the gelatin capsule after eclosion frequently resulted in aphid appendages being broken from the mummy. The emergence lid frequently became detached from the mummy either by the parasite chewing an entire circle with its mandibles during eclosion or by the parasite breaking the short attachment section either during the eclosion process or during the post-eclosion activity inside the gelatin capsule. It was considered essential to adjust the weights of the incomplete mummies for the weights of their missing parts since the weights of these missing parts were not trivial when compared to the standard deviation of the mummy weights.

4.3.2 Parasite weight variance analysis: results

Tables 5 and 6 present the 4-way (sex, host age, experiment number, cage number) partially hierarchal

Figure 2. Relationship between the weight of a mummy and the weight of its various appendages and emergence lid.

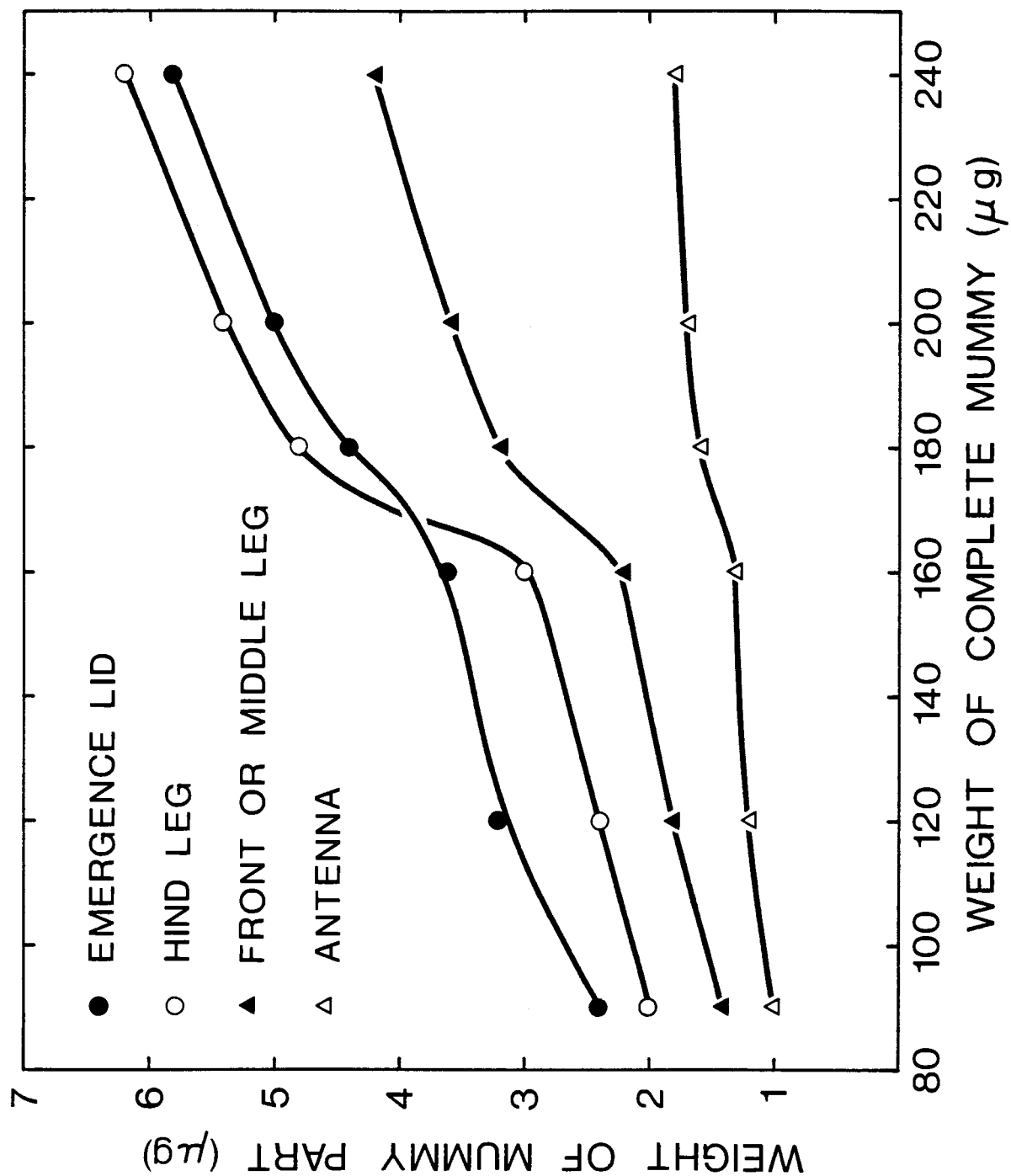

EMERGENCE LID
HIND LEG
FRONT OR MIDDLE LEG
ANTENNA
WEIGHT OF MUMMY PART (μg)
WEIGHT OF COMPLETE MUMMY (μg)
1
2
3
4
5
6
7
80
100
120
140
160
180
200
220
240

Table 5 A 4-way (sex, host age, experiment number, cage number) partially hierarchal ANOVA of Aphidius smithi pupa weight (μg) 9 days after oviposition.

Source of variation	DF	SS	MS	F	Probability	Expected[a] mean square	Denominator MS for F
Sex (A)	1	386643	386643	132.60	.05<p<.10	$\sigma_\varepsilon^2 + nqrs\sigma_\alpha^2 + nqs\sigma_{\alpha\gamma}^2 + n\sigma_{\alpha\delta}^2$	AC
Host age (B)	11	5909697	537245	78.73	p<.001	$\sigma_\varepsilon^2 + nprs\sigma_\beta^2 + nps\sigma_{\beta\gamma}^2 + np\sigma_\delta^2$	BC
AB interaction	11	45691	4154	0.95	.50<p<.75	$\sigma_\varepsilon^2 + nrs\sigma_{\alpha\beta}^2 + ns\sigma_{\beta\gamma}^2 + n\sigma_{\alpha\delta}^2$	ABC
Experiment number (C)	1	37409	37409	2.54	.10<p<.25	$\sigma_\varepsilon^2 + npqs\sigma_\gamma^2 + np\sigma_\delta^2$	D
AC interaction	1	2916	2916	1.24	.25<p<.50	$\sigma_\varepsilon^2 + nqs\sigma_{\alpha\gamma}^2 + n\sigma_{\alpha\delta}^2$	AD
BC interaction	11	75064	6824	0.46	p>.75	$\sigma_\varepsilon^2 + nps\sigma_{\beta\gamma}^2 + np\sigma_\delta^2$	D
ABC interaction	11	47970	4361	1.86	p≈0.058	$\sigma_\varepsilon^2 + ns\sigma_{\alpha\beta\gamma}^2 + n\sigma_{\alpha\delta}^2$	AD
Cage number (D)	72	1058573	14702	6.12	p<.001	$\sigma_\varepsilon^2 + np\sigma_\delta^2$	error
AD interaction	72	168811	2345	0.98	.50<p<.75	$\sigma_\varepsilon^2 + n\sigma_{\alpha\delta}^2$	error
Error	1026	11602410	2401			σ_ε^2	

a) n=4.71=harmonic mean of the number of observations per cell
p=2=number of sexes
q=12=number of host ages
r=2=number of experiments
s=4=number of cages

Table 6 A 4-way (sex, host age, experiment number, cage number) partially hierarchal ANOVA of *Aphidius smithi* post eclosion mummy weight (μg).

Source of variation	DF	SS	MS	F	Probability	Expected[a] mean square	Denominator MS for F
Sex (A)	1	4978	4978	137.99	$.05<p<.10$	$\sigma_\varepsilon^2 + nqrs\sigma_\alpha^2 + nqs\sigma_{\alpha\gamma}^2 + ns\sigma_{\alpha\delta}^2$	AC
Host age (B)	11	282627	25693	91.55	$p<.001$	$\sigma_\varepsilon^2 + nprs\sigma_\beta^2 + nps\sigma_{\beta\gamma}^2 + np\sigma_\delta^2$	BC
AB interaction	11	1626	148	1.00	$.50<p<.75$.	$\sigma_\varepsilon^2 + nrs\sigma_{\alpha\beta}^2 + ns\sigma_{\alpha\beta\gamma}^2 + n\sigma_{\alpha\delta}^2$	ABC
Experiment number (C)	1	941	941	2.54	$.10<p<.25$	$\sigma_\varepsilon^2 + npqs\sigma_\gamma^2 + np\sigma_\delta^2$	D
AC interaction	1	36	36	0.49	$.25<p<.50$	$\sigma_\varepsilon^2 + nqs\sigma_{\alpha\gamma}^2 + n\sigma_{\alpha\delta}^2$	AD
BC interaction	11	3087	281	0.76	$.50<p<.75$	$\sigma_\varepsilon^2 + nps\sigma_{\beta\gamma}^2 + np\sigma_\delta^2$	D
ABC interaction	11	1630	148	2.00	$p\approx0.040$	$\sigma_\varepsilon^2 + ns\sigma_{\alpha\beta\gamma}^2 + n\sigma_{\alpha\delta}^2$	AD
Cage number (D)	72	26669	370	4.61	$p<.001$	$\sigma_\varepsilon^2 + np\sigma_\delta^2$	error
AD interaction	72	5333	74	0.92	$.50<p<.75$	$\sigma_\varepsilon^2 + n\sigma_{\alpha\delta}^2$	error
Error	1026	388395	80			σ_ε^2	

a) n=4.71=harmonic mean of the number of observations per cell
p=2=number of sexes
q=12=number of host ages
r=2=number of experiments
s=4=number of cages

ANOVA of A. smithi pupa weight and mummy weight respectively. Table 7 presents the 3-way (sex, host age, cage number) partially hierarchal ANOVA of A. smithi adult weight. These ANOVA tables are interpreted as follows:

- Rearing the parasites in more than one cage (factor D) resulted in a significant additional variance component in all three weight variates.
- The relationship between the sexes depends upon the cage number (AD interaction) in the adults, but not in the pupae or mummies.
- There may be an interaction between the sex, the host age and the experiment number (ABC interaction) in pupa weight and mummy weight (the variance ratio is too close to the critical F for an accurate interpretation).
- The relationship between the host ages is reproducible (BC interaction) in pupa weight and mummy weight.
- The relationship between the sexes is reproducible (AC interaction) in pupa weight and mummy weight.
- Duplicating the experiment (factor C) did not result in an additional variance component in pupa weight or mummy weight.

Table 7 A 3-way (sex, host age, cage number) partially hierarchal ANOVA of Aphidius smithi adult weight (μg) after dessication

Source of variation	DF	SS	MS	F	Probability	Expected[a] mean square	Denominator MS for F
Sex (A)	1	13533	13533	376.97	$p<.001$	$\sigma_\varepsilon^2 + nqs\sigma_\alpha^2 + n\sigma_{\alpha\delta}^2$	AD
Host age (B)	11	28597	2600	14.78	$p<.001$	$\sigma_\varepsilon^2 + nps\sigma_\beta^2 + np\sigma_\delta^2$	D
AB interaction	11	536	49	1.36	$.10<p<.25$	$\sigma_\varepsilon^2 + ns\sigma_{\alpha\beta}^2 + n\sigma_{\alpha\delta}^2$	AD
Cage number (D)	36	6332	176	7.68	$p<.001$	$\sigma_\varepsilon^2 + np\sigma_\delta^2$	error
AD interaction	36	1291	36	1.57	$p\approx0.020$	$\sigma_\varepsilon^2 + n\sigma_{\alpha\delta}^2$	error
Error	553	66242	23			σ_ε^2	

a) n=5.24= harmonic mean of the number of observations per cell
p=2= number of sexes
q=12= number of host ages
s=4= number of cages

- The relationship between the sexes does not depend upon the age of the host (AB interaction) in any of the three weight variates.
- Parasites reared on different ages of host aphid (factor B) are significantly different in all three weight variates.
- Females are significantly heavier than males (factor A) in all three weight variates.

Initial analysis of the sex factor (factor A) revealed differences in the sexes in adult weight (Table 7), but not in pupa weight (Table 5) or mummy weight (Table 6). The F-test for pupa weight and mummy weight is not very powerful due to the AC interaction denominator. Denominators consisting of interactions between random and fixed factors and having low degrees of freedom cannot yield powerful F-tests. A more powerful denominator can be obtained by dropping non-significant expected mean square terms from the model and pooling their mean squares (weighted by their degrees of freedom) (Winer 1971). Using this procedure for both pupa weight and mummy weight, the $nd^2_{\alpha\delta}$ term drops out of the model (the AD interaction is nonsignificant). The expected mean square for the sex factor becomes $\sigma^2_{\varepsilon'} + nqrs\sigma^2_{\alpha}$ where $\sigma^2_{\varepsilon'}$ is the pooled weighted mean square. The appropriate denominator mean square for

the F-test becomes 2398 for pupa weight and 79.6 for mummy weight, each with 1099 degrees of freedom. The F-ratios are 16.11 (pupa weight) and 62.54 (mummy weight), both of which are highly significant. Thus, female parasites are significantly heavier than male parasites in all three weight variates.

Figs. 3, 4 and 5 show the relationship between the dry weight of the host aphid at oviposition and the pupa weight, mummy weight and adult weight of the parasite respectively. There are several reasons why host dry weight at oviposition was chosen for the independent axis rather than host age at oviposition. Host dry weight at oviposition is more indicative of the quantity of food available for the parasite. Within limits, host dry weight will be a function of the physiological age of the host. Although the relationships in Figs 3, 4 and 5 are certainly not applicable to aphids reared under extreme environments, they should be valid for aphids reared under environments only slightly different from that used in this experiment, such as temperatures of 18°C or 22°C. Thus, host dry weight at oviposition is less restrictive with respect to the environmental conditions under which the host were reared. The relationship between aphid age and aphid dry weight

Figure 3. Relationship between host dry weight at oviposition (μg) and male and female Aphidius smithi pupa wet weight (μg) nine days after oviposition.

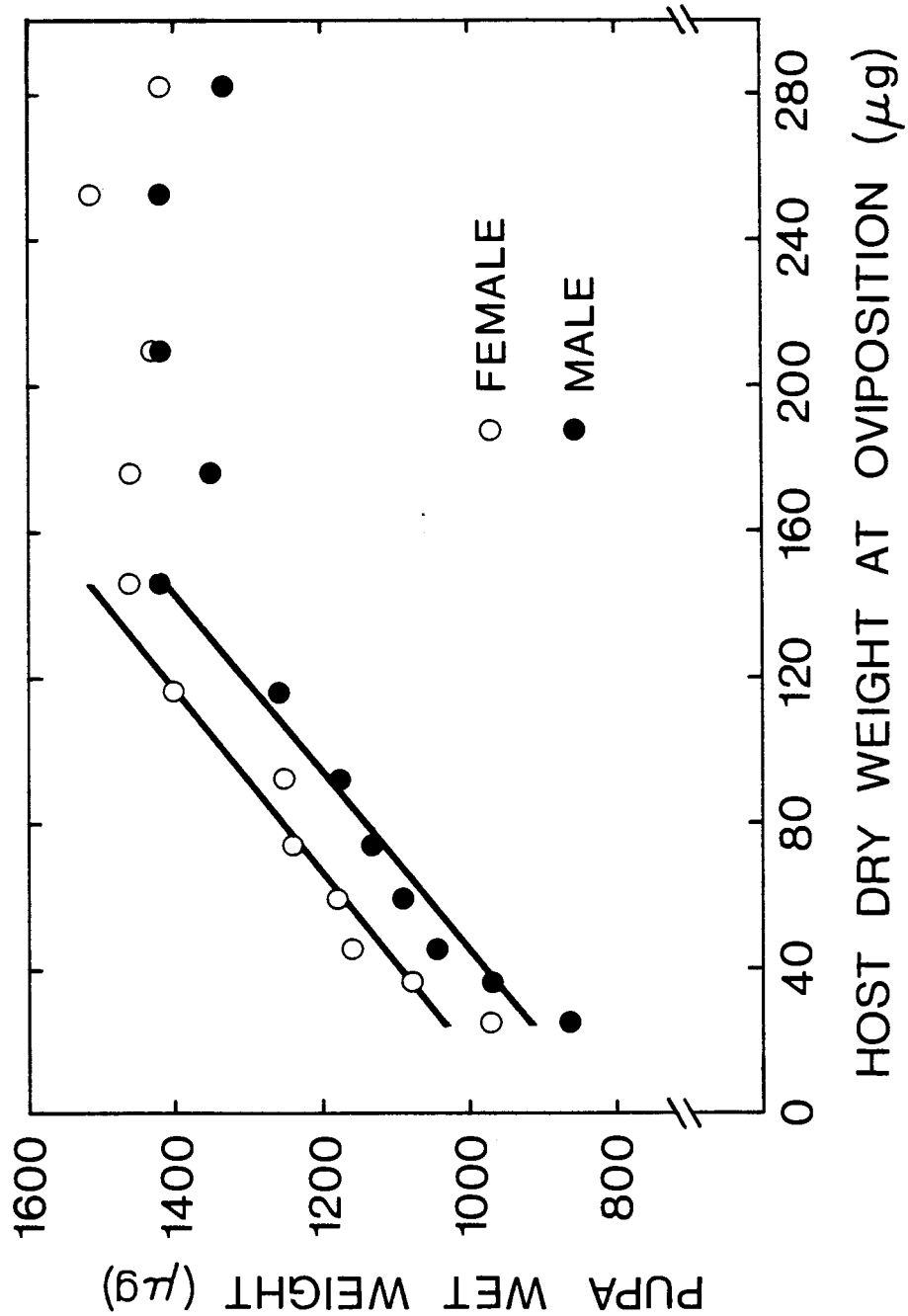

PUPA WET WEIGHT (μg)
1600
1400
1200
1000
800
0
40
80
120
160
200
240
280
HOST DRY WEIGHT AT OVIPOSITION (μg)
FEMALE
MALE

Figure 4. Relationship between host dry weight at oviposition (µg) and male and female Aphidius smithi vacated mummy weight (µg).

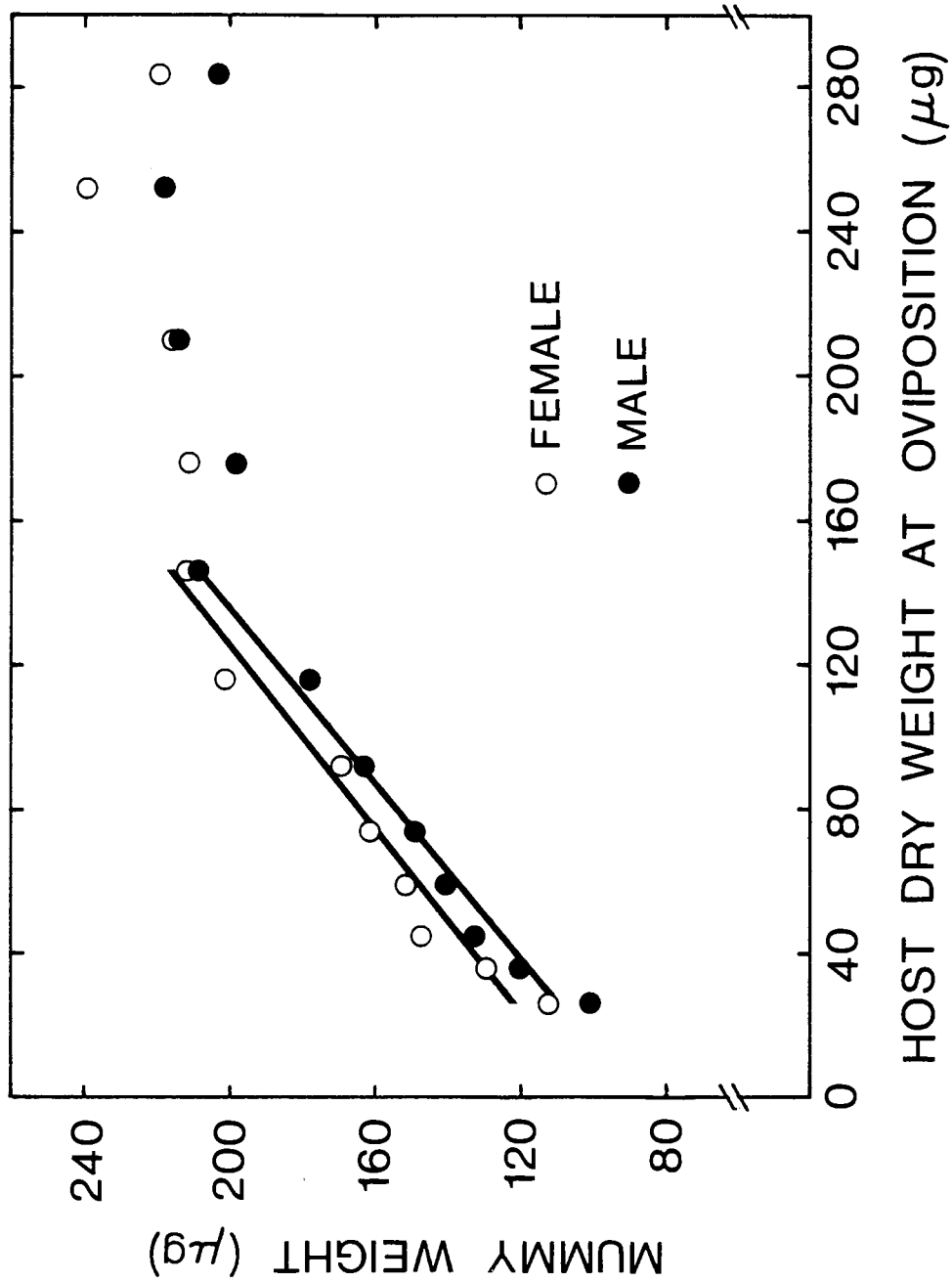
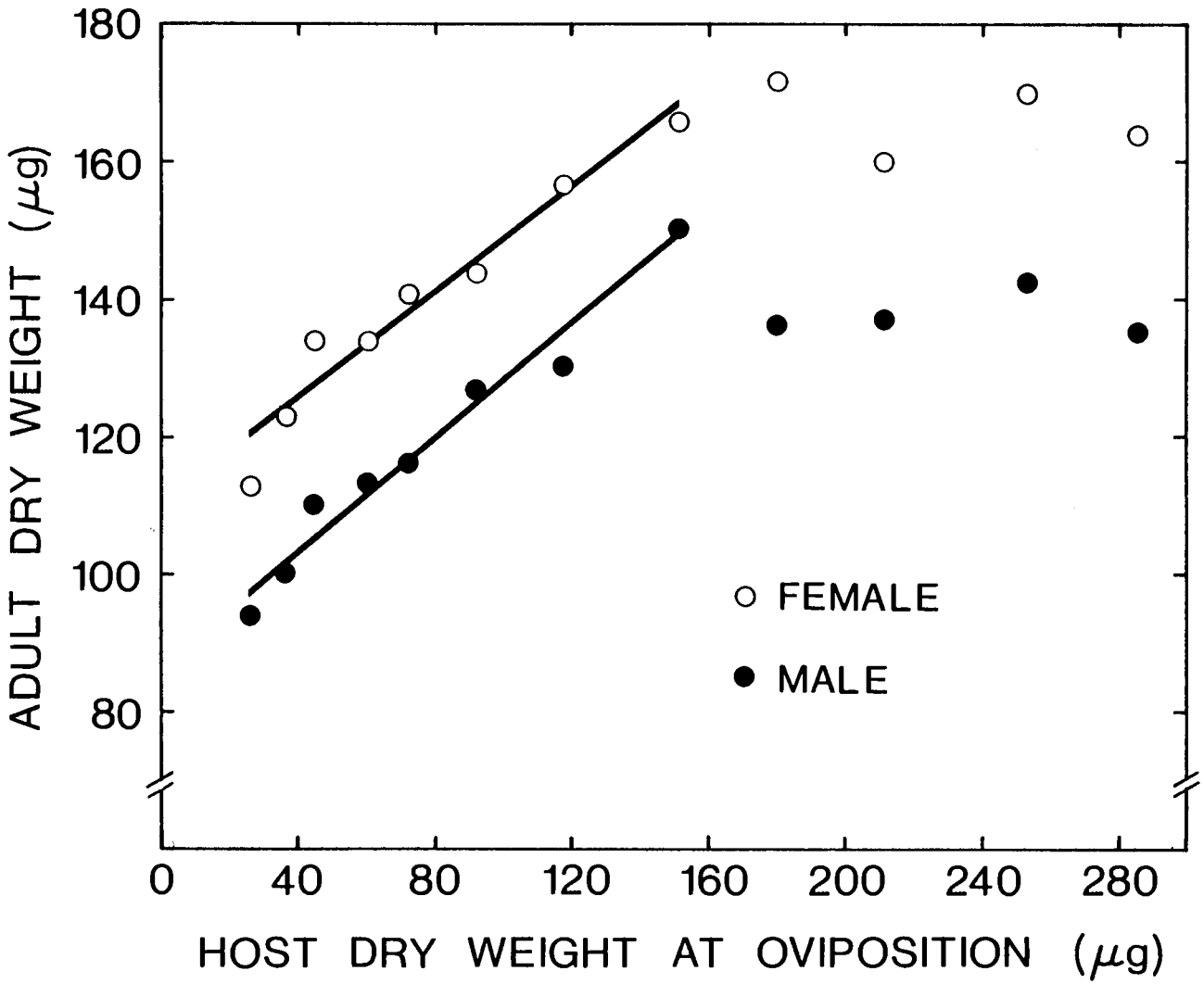

MUMMY WEIGHT (μg)
240
200
160
120
80
0
40
80
120
160
200
240
280
HOST DRY WEIGHT AT OVIPOSITION (μg)
FEMALE
MALE

Figure 5. Relationship between host dry weight at oviposition (μg) and male and female Aphidius smithi adult dry weight (μg) after desiccation.


180
160
140
120
100
80
ADULT DRY WEIGHT (μg)
0
40
80
120
160
200
240
280
HOST DRY WEIGHT AT OVIPOSITION (μg)
FEMALE
MALE

should be constant for any set of environmental parameters. This relationship, for the parameters used in this experiment, is shown in Fig. 6 (data obtained by weighing a sample of host aphids from each ovipositional session).

The mean parasite weights in Figs. 3, 4 and 5 were obtained by pooling over cage number and experiment number for each of the host age groups. Pooling over experiment number is justifiable since the variance component due to multiple experiments was not significant (factor C in Tables 5 and 6). Pooling over cage number, even though the multiple cage variance component was significant (factor D in Tables 5, 6 and 7) is justifiable since this random variance component can be considered as part of the experimental error.

The regression lines in Figs. 3, 4 and 5 were calculated using analysis of variance with regression (Sokal and Rohlf 1969) on host weights between 25.84q (=0.5 days old) at oviposition and 146.54q (=4.0 days old) at oviposition. The regression tables are presented in Table 8 for pupa weight, Table 9 for mummy weight and Table 10 for adult weight. The regression statistics are presented in Table 11. The obvious effects of host age and sex in Figs. 3, 4 and 5 support the statistical significance of the host age and sex

Figure 6. The relationship between age and dry weight in the pea aphid, Acyrthosiphon pisum, reared under the environmental conditions of the study.

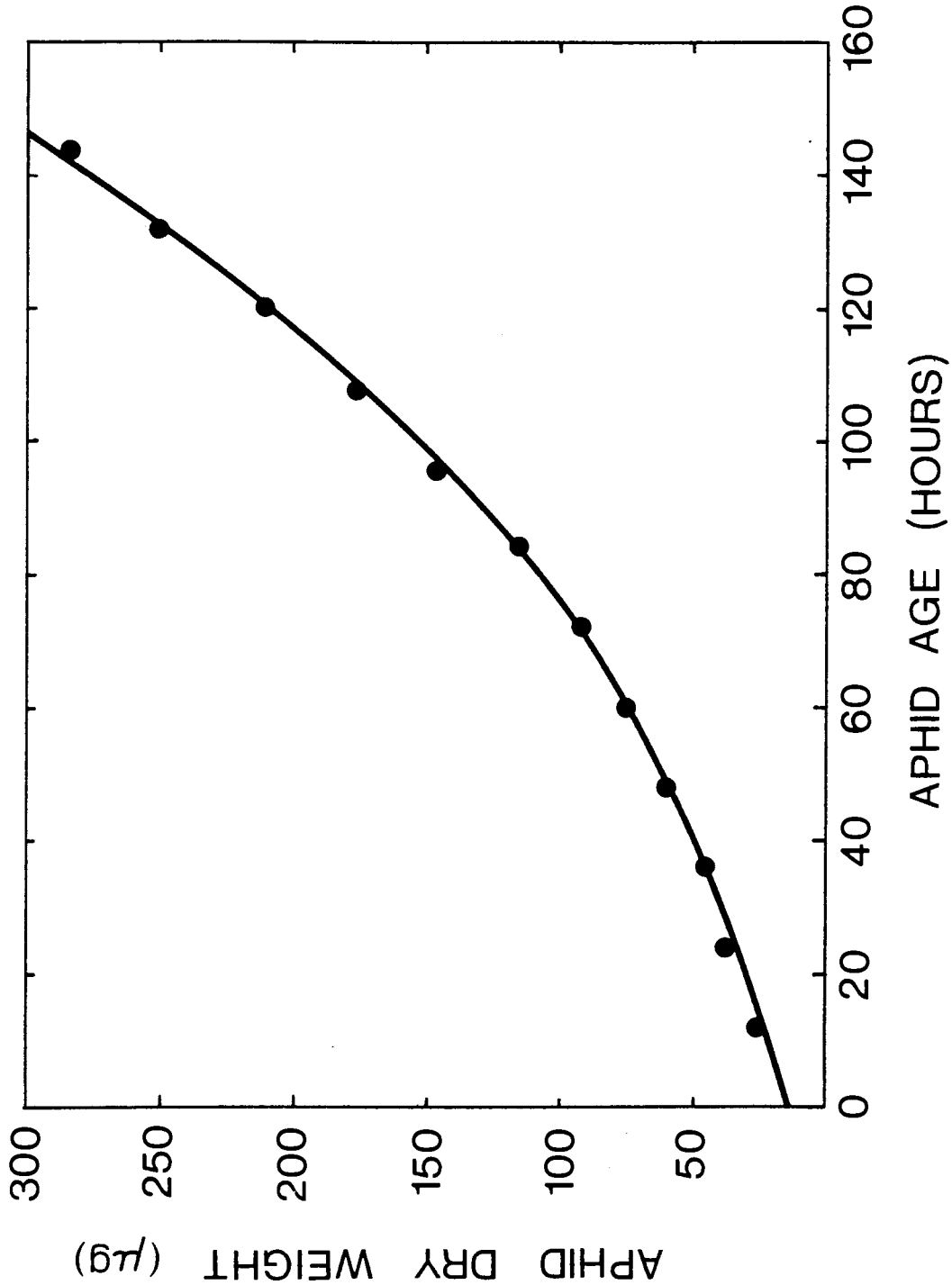

APHID DRY WEIGHT (µg)
300
250
200
150
100
50
0
20
40
60
80
100
120
140
160
APHID AGE (HOURS)

Table 8 An analysis of variance of Aphidius smithi pupa weight (μg) regressed on host weight (μg) at oviposition for hosts between 25.8μg (0.5 days) and 146.5μg (4.0 days). A) Males. B) Females.

A)

Source of variation	DF	SS	MS	F	
Among host weight groups	7	11454460	1636352	137.52	***
Linear regression	1	10852870	10852870	108.24	***
Deviations from regression	6	601594	100266	8.43	***
Error	519	6175488	11899		

B)

Source of variation	DF	SS	MS	F	
Among host weight groups	7	76665408	1095058	69.87	***
Linear regression	1	7249152	7249152	104.49	***
Deviations from regression	6	416256	69376	4.43	***
Error	280	4388096	15672		

*** $p<0.001$

Table 9 An analysis of variance of *Aphidius smithi* mummy weight (μg) regressed on host weight (μg) at oviposition for hosts between 25.8μg (0.5 days) and 146.5μg (4.0 days). A) Males. B) Females.

A)

Source of variation	DF	SS	MS	F	
Among host weight groups	7	452493	64642	181.74	***
Linear regression	1	440503	440503	220.44	***
Deviations from regression	6	11990	1998	5.62	***
Error	519	184596	356		

B)

Source of variation	DF	SS	MS	F	
Among host weight groups	7	335801	47972	106.39	***
Linear regression	1	323122	323122	152.91	***
Deviations from regression	6	12679	2113	4.69	***
Error	280	126257	451		

*** $p<0.001$

Table 10 An analysis of variance of Aphidius smithi adult weight (μg) regressed on host weight (μg) at oviposition for hosts between 25.8μg (0.5 days) and 146.5μg (4.0 days). A) Males. B) Females.

A)

Source of variation	DF	SS	MS	F	
Among host weight groups	7	56942	8135	74.57	***
Linear regression	1	54972	54972	167.40	***
Deviations from regression	6	1970	328	3.01	***
Error	252	27488	109		

B)

Source of variation	DF	SS	MS	F	
Among host weight groups	7	50323	7189	39.00	***
Linear regression	1	48523	48523	161.74	***
Deviations from regression	6	1800	300	1.63	n.s.
Error	163	30047	184		

n.s. not significant; *** $p<0.001$

TABLE 11 Regression statistics for *Aphidius smithi* weight (μg) regressed on host weight (μg) at oviposition for hosts between 25.8μg (0.5 days) and 146.5μg (4.0 days).

Variate	Sex	Regression coefficient	y intercept	Standard error regression coefficient	95% confidence limits for the regression coefficient	
					Lower	Upper
Pupa weight	Males	4.05	817.20	0.39	3.10	5.10
Pupa weight	Females	3.96	938.18	0.37	2.86	4.66
Mummy weight	Males	0.82	88.31	0.055	0.68	0.95
Mummy weight	Females	0.79	101.31	0.064	0.64	0.95
Adult weight	Males	0.417	86.43	0.032	0.338	0.496
Adult weight	Females	0.379	110.82	0.030	0.306	0.453

factors in Tables 5, 6 and 7.

Linear regression removed a very significant proportion of the variation in pupa weight, mummy weight and adult weight (Tables 8, 9 and 10) in both males and females in hosts between 25.8µg (=0.5 days old) at oviposition and 146.5µg (=4.0 days old) at oviposition. Unfortunately, except for adult weight in the females (Table 10B), there is also very significant heterogeneity about the regression lines, as is evident from Figs. 3, 4 and 5.

Linear regression did not remove a significant proportion of the variation in pupa weight, mummy weight or adult weight in either males or females in hosts between 146.5µg (=4.0 days old) at oviposition and 282.9µg (=6.0 days old) at oviposition (analysis not presented).

4.3.3 Parasite weight variance analysis: discussion

The additional variance component caused by rearing the developing parasites in separate cages (the cage number source of variation in Tables 5, 6 and 7) is a serious source of experimental error in this experimental system. The parasitized aphids were randomly placed into the four cages in each ovipositional session. The only possible differences between the cages are minor environmental differences,

such as host plant quality, temperature, humidity and level of crowding. It is well known that the weight of an adult insect is dependent upon the environment it experiences during its development (Chapman 1969). The weight of the host aphid used in these experiments is known to be affected by temperature and crowding (Murdie 1969). In this analysis, this variance component was removed by replication over cages, but it was not possible to include this replication factor in the subsequent experiments. In Chapters 5 and 6 all the offspring of a single female were reared in the same cage. Because of this significant cage number variance component, the difference between cages cannot be attributed to genetic factors alone. This has certainly reduced the efficiency of the selection program (Chapter 6). Additional research on the effect of environmental parameters on parasite weight would be informative.

The significant sex-cage number interaction in adult weight (Table 7) indicates that environmental differences between cages affect male and female adult weights differently. With the present data, there is no way of determining which environmental factor(s) may have caused the interaction since cage number is a random factor. There is a possibility that this

interaction is a consequence of the nonorthogonality in the sources of variance, since nonorthogonal sources of variance are not independent (Gilbert 1973, Winer 1971). More extensive data with fixed orthogonal environmental factors are needed for a precise interpretation.

The size difference between the two sexes is probably a consequence of the different reproductive functions of males and females. Price (1972) similarly observed larger females than males in two Ichneumonid parasitoids of Neodiprion swainei. Because of their larger size, female larvae may require more food for development than male larvae.

Definite trends are apparent in the relationships between host weight at oviposition and parasite weight (Figs. 3, 4 and 5). Increases in the weight of the host aphid at oviposition result in corresponding increases in the weight of the parasite emerging from the aphid up to a host weight of 146.54g. Linear regression removed a very significant proportion of the variation in parasite weight from these lighter hosts (Tables 8, 9 and 10). Further increases in the weight of the host aphid beyond 146.54g at oviposition did not result in further increases in the weight of the parasite emerging from the aphid.

After oviposition, the host aphid will continue to feed and develop for 5 or 6 days before being killed by the parasite larva (Cloutier 1978). The quantity of food available for the developing parasite will depend upon the size of the host aphid during parasite develcpment, which in turn is largely dependent upon the age or weight of the host aphid at oviposition. The data presented in this chapter indicate that in host aphids less than approximately 1464g in weight at oviposition, growth and weight in the parasite will be limited by the amount of food available in the host aphid. In these small hosts, parasite growth is probably being restricted by the amount of food available at specific points during the development of the parasite larva. Perhaps certain points are more critical than others. Aphid-parasite bioenergetics has been examined (Cloutier 1978), but because of the biological nature of the food source, it is not possible to control the quality or quantity of food at each stage during parasite development. Valuable information could be obtained if it were possible to rear the parasite within a synthetic diet.

A parasite larva oviposited into a small host could compensate for the reduced quantity of food by stimulating host feeding or by postponing the time at

which the host is killed. Cloutier (1978) observed higher feeding rates in the parasitized aphids than in unparasitized aphids. A delay in killing the host would result in an increased developmental time. Smith (published in Mackauer 1973) found that A. smithi did take longer to develop in first instar hosts than in second instar hosts, but he also found that the parasite took longer to develop in third instar hosts than in second instar hosts. Perhaps delays in killing the host only occur with very small host aphids. Postponing the killing of the host may not be a common phenomenon since fitness gained by increasing size may be lost by the increased developmental time. Unfortunately it was not possible to record develcpmental time in the experiments presented in this chapter.

There are several possible reasons for the levelling off of parasite weights in hosts heavier than 146.54g at oviposition. Heavier hosts may not provide an increase in the quantity of food available to the parasite larva since these heavier aphids have reached their full growth capacity before parasite mummification, and since much of the nutrient buildup in these heavier aphids is being used for aphid reproduction. There may also be a genetically

determined weight limit in A. smithi. Heavier parasite larva may be physically too large to develop inside an aphid without destroying or interfering with aphid tissue essential for aphid feeding. The heterogeneity of the parasite weights in these heavier host aphids may be caused by crowding from the offspring produced by these aphids before they mummify, or it may be caused by heterogeneity in some other environmental or genetic factor.

Since natural populations contain unparasitized aphids of all weights, and since parasite weight is greatly influenced by the weight of the host aphid at oviposition, host aphid weight variation is a significant cause of parasite weight variation in natural populations. Moreover, with knowledge of aphid age structures in natural populations it may be possible to determine adult parasite host preferences from the distributions of parasite weights found in these natural populations.

4.3.4 Relationships between the approaches to measuring weight

In this chapter, the weight of each individual parasite was measured using three different approaches, namely weighing the pupa, weighing the mummy and weighing the adult. It was proposed, in Chapter 3, that

the variates obtained from each approach are highly correlated with a hypothetical parasite weight variate. This proposal was based on data from 2-day-old host aphids. It is desirable to have a measurement technique for parasite weight that can be used with any age of host aphid. Thus, it was felt that data should be provided on the correlation between all three variates from a larger range of host aphid ages.

The correlations between pupa (wet) weight, mummy weight and adult (dry) weight for each host age are presented in Table 12. All correlations are highly significant. Unfortunately, it was not possible to correlate the variates for each age of host aphid with a more accurate variate, such as wet weight 1 hour after eclosion since it was not possible to measure this variable in such a large experiment. However, the high correlation between the variates in all host ages suggests that all three approaches to measuring parasite weight can be used with host aphids ranging in age from 0 to 6 days.

The relationships between the three weight variates is one of association, not of dependence. Thus, it is not possible to convert data from one variate to another. However, because of the high correlation (association) between the variates, the

Table 12 Correlations between Aphidius smithi pupa weight, mummy weight and adult weight for parasites oviposited into various ages of pea aphid, Acyrthosiphon pisum.

Host age at oviposition (days)	Pupa weight-mummy weight				Pupa weight-adult weight				Mummy weight-adult weight			
	males		females		males		females		males		females	
	r	N	r	N	r	N	r	N	r	N	r	N
0.5	0.942	72	0.932	36	0.997	38	0.995	24	0.958	38	0.973	24
1.0	0.873	84	0.955	27	0.968	41	0.974	17	0.918	41	0.948	17
1.5	0.869	72	0.921	37	0.975	37	0.927	23	0.821	37	0.850	23
2.0	0.896	79	0.960	27	0.770	43	0.977	16	0.765	43	0.974	16
2.5	0.901	70	0.816	26	0.970	39	0.933	10	0.952	39	0.830	10
3.0	0.828	56	0.971	31	0.992	27	0.995	13	0.863	27	0.973	13
3.5	0.937	54	0.903	54	0.859	16	0.942	40	0.858	16	0.849	40
4.0	0.930	40	0.902	51	0.777	19	0.809	28	0.828	19	0.767	28
4.5	0.971	49	0.923	45	0.991	19	0.974	28	0.976	19	0.922	28
5.0	0.896	57	0.920	55	0.873	27	0.956	40	0.821	27	0.935	40
5.5	0.888	44	0.906	62	0.974	20	0.954	35	0.894	20	0.883	35
6.0	0.945	39	0.825	51	0.977	25	0.884	24	0.951	25	0.860	24

results of experiments in which one variate was measured can be extrapolated to the other variates. This is supported by the similar relationships between host age and parasite weight for each of the variates (Figs. 3, 4 and 5).

4.4 SUMMARY

Analysis of parasite weight variance, using pupa weight 9 days after oviposition, empty (vacated) mummy weight and oven dried adult weight, revealed the following in each of the above variates (with exceptions as noted): The weights of the parasites resulting from oviposition into host aphids ranging in weight (age) from 26µg (0.5 days old) to 238µg (6.0 days old) were found to be heterogeneous. Parasite weight increased as the weight (age) of the host at oviposition increased from 26µg (0.5 days) to 146µg (4.0 days). Further increases in host weight at oviposition did not result in further increases in parasite weight. Linear regression of parasite weight on host weight at oviposition removed a significant proportion of the parasite weight variance between host weight (age) groups with hosts between 26µg (0.5 days) and 146µg (4.0 days), but there was significant heterogeneity about the regression lines (with the exception of adult weight in females). Male parasite weight was found to be significantly less than female parasite weight. An additional parasite weight variance component was introduced by environmental differences between cages within the host weight (host age) groups. An additional parasite weight variance component was

not introduced by repeating the experiment. Sex-cage number interaction in adult weight was the only factor interaction that was significant.

The correlations between pupa weight, mummy weight and adult weight were found to be significant for each sex within each host weight (host age) group.

CHAPTER 5

THE VARIATION OF PARASITE WEIGHTS IN TWO FIELD POPULATIONS OF APHIDIUS SMITHI IN BRITISH COLUMBIA

5.1 INTRODUCTION

In recent years, new interest has been generated in studies on variation between and among populations. Electrophoretic and morphometric studies have demonstrated that variation is the rule rather than the exception (Gould and Johnston 1972).

The greatest difficulty in the study of geographic variation is the determination of the causes of the variation (Gould and Johnston 1972). Genetic variation cannot be implied from phenotypic variation, especially with quantitative characters. One of the ways to study geographic variation is to minimize the environmental component of the phenotypic variation by rearing individuals in a constant laboratory environment.

Sokoloff (1965) and Stalker and Carson (1947) used this approach to study variation in Drosophila populations. Similar methodology will be used in this chapter to study the variation between field populations of A. smithi.

A. smithi was originally described from India by Sharma and Subba Rao (1959). In 1958 breeding stocks of the parasite were brought into the United States for mass rearing and subsequent release in the eastern and western United States for the biological control of the pea aphid. A. smithi was first recorded in British Columbia in 1965 near Christina Lake, probably having migrated from release sites in the western United States (Mackauer and Finlayson 1967). The parasite is thought to have spread into the Kamloops area sometime between 1966 and 1968, since well established populations were found in Kamloops in 1969 (Campbell and Mackauer 1973). It was recorded near Ashcroft in 1971 by Campbell (1973). In the Kamloops-Ashcroft region, established populations of A. smithi are restricted mainly to commercial alfalfa fields, where they are the most abundant primary parasite of the pea aphid (Campbell and Mackauer 1973).

In this chapter, A. smithi populations from Kamloops and Ashcroft are examined for within and

between population variation in adult weight. Knowledge gained from the study of weight variation in the laboratory (Chapters 3 and 4) is used to describe and interpret weight variation found in the field populations.

5.2 MATERIALS AND METHODS

Samples of mummified aphids were collected on July 7, 1976 from two alfalfa fields, one near Ashcroft, B.C. and the other located on the grounds of the Agriculture Canada Research Station, Kamloops, B.C. The alfalfa was mature in both fields with over 50% of the plants in bloom. Each of the fields was square in shape and approximately 5 acres in size. The fields were subdivided (by eye) into 5 parallel rectangular sections. A longitudinal transverse was made through the middle of each of these sections, collecting all the mummies that were observed. All surfaces of the plants were inspected. Within 12 hours after collection, the mummified aphids were placed into gelatin capsules, which were kept in a growth chamber at 20°C. The mummified aphids potentially contained one of three species of primary parasites or one of several species of secondary parasites (Campbell 1973; Mackauer and Campbell 1972). All parasites but A. smithi were removed from the field samples either in the mummy stage (Praon pupates beneath the eviscerated mummy) or upon emergence (the secondary parasites have a distinct behavior and morphology; the thorax of Aphidius ervi is darker than that of A. smithi).

A laboratory reared F_1 generation consisting only

of males was obtained for each location by allowing 16 virgin female parasites from Kamloops and 44 virgin female parasites from Ashcroft to oviposit into 48±2.5-hour-old (second instar) host aphids. Twelve parasites (some from Kamloops and some from Ashcroft) were used in each of 5 one-hour ovipositional sessions. Each female was allowed to lay one egg into each of 10 aphids, and these 10 aphids were transferred to a plant in a separate plastic cage and allowed to develop. The mummies containing the developing parasites were removed from the plant surface 8 days after oviposition and placed into gelatin capsules. A random sample of 10 control aphids was taken at the beginning of each ovipositional session and oven-dried for subsequent determination of the mean host weight at oviposition.

Of the three parasite weight variates used in the previous chapter, oven-dried parasite weight was the only one that was technically feasible to use with field-collected parasites. Pupa weight 9 days after oviposition cannot be determined for field samples since the time of oviposition is unknown; also, environmental heterogeneity would introduce considerable variation into the physiological ages of the host and parasite at 9 days after oviposition. Although mummy weights can be measured in field

populations, these measurements are subject to error since field mummies tend to be heavily damaged, and thus require considerable adjustment for missing aphid appendages. Oven-dried adult weight is both independent of the time of oviposition and not affected by missing aphid appendages in the mummy.

The field collected parasites and their F_1 offspring were kept in gelatin capsules until 6-8 days after death, when they were oven-dried for 4-5 days and weighed. This weighing procedure is identical to that used for determining adult weights in Chapter 4.

The variance of adult weights in the F_1 males (produced by females from the two field populations) was analyzed using a 2-way nested ANOVA. The model used was:

$$\overline{AB}_{ij} = \mu + \alpha_i + \beta_{j(i)} + \overline{\varepsilon}_{ij}$$

where:

$\overline{AB}_{ij}$ = the mean weight in cell ij

μ = the parametric grand mean

α_i = the effect of location (i.e. Kamloops vs. Ashcroft)

$\beta_{j(i)}$ = the effect of the cage (since the offspring from each parent were placed into separate cages, this is the mean effect of the parent plus the effect of the unique environment

in each cage)

$\bar{\epsilon}_{ij}$ = the mean experimental error in cell ij

5.3 RESULTS AND DISCUSSION

The Ashcroft sample included 346 mummies, from which 103 female and 77 male A. smithi emerged. The Kamloops sample included 138 mummies, from which 35 female and 25 male A. smithi emerged. Descriptive statistics of the adult weights from these populations are presented in Table 13. One-way ANOVA on the field samples showed a significant difference between Ashcroft and Kamloops male weight ($F=15.2$, $p<0.001$) and female weight ($F=14.7$, $p<0.001$). Thus, there is geographic variation in weight between the Ashcroft and Kamloops populations.

Descriptive statistics of the laboratory-reared male offspring of the field collected females are also presented in Table 13. The mean weight (±S.E.) of the aphids used as hosts for these male offspring was 69.5±1.0μg. Using the regression equation from Table 11, the 95% confidence limits on mean male parasite weight from this weight of host aphid are 109.9μg and 120.9μg; male offspring weights in Table 13 are within these confidence limits.

The 2-way nested ANOVA on the F_1 males from the field collected parasites (Table 14) indicates that the male weights from the two populations are statistically indistinguishable when reared in a constant laboratory

Table 13 Descriptive statistics on adult dry weight (μg) of *Aphidius smithi*. A) Field samples collected in Ashcroft and Kamloops. B) Male offspring from field samples collected in Ashcroft and Kamloops.

	Population	Sex	N	Mean	S.E.	Median	C.V.
A)	Ashcroft	M	77	110.00	2.80	108.00	22.2
	Ashcroft	F	103	127.93	2.54	123.00	20.2
	Kamloops	M	25	88.56	4.40	86.00	24.8
	Kamloops	F	35	108.37	4.54	109.00	24.8
B)	Ashcroft F_1	M	365	119.04	0.67	118.86	10.8
	Kamloops F_1	M	107	113.93	1.30	113.91	11.8

Table 14 A 2-way nested ANOVA of Aphidius smithi adult weight (µg) using F_1 male offspring of field collected females.

Source of Variation	DF	SS	MS	F	
Location	1	2450	2450	3.57	n.s.
Cage number	58	39837	687	6.64	***
Error	412	42630	103		

n.s. not significant; *** $p<0.001$

environment. Weight variance between the populations in the field environments will be much greater than weight variance between the populations in a constant laboratory environment. Although the genetic component of weight variance may be slightly larger in the field environments (due to genotype-environment interaction), it is very unlikely that it will increase as much as the environmental component of weight variance. Since the genetic component of weight variance between the two populations was not detectable when the environmental component was minimized (in the laboratory), it is even less likely to be detectable when the relative contribution of the environmental component is increased (in the field environments). Samples taken from field populations showed geographic variation in parasite weight between Ashcroft and Kamloops. Thus, this variation is caused by differences in the environment between Ashcroft and Kamloops.

There are many possible reasons for the lack of obvious genetic differentiation between the Kamloops and Ashcroft populations. The populations are less than 100km apart. There is potential for gene flow along populations on the Thompson River which flows near both the Ashcroft and Kamloops populations. A highway which passes near both populations is frequently used

as a trucking route for baled alfalfa, which could easily have mummies on its surface. Also, considering the origin of A. smithi in B.C. , it is not unreasonable to assume that the two populations have only separated recently, and that the ancestral population was not highly variable.

There are many environmental parameters which could have contributed towards producing the observed parasite weight differences between the Ashcroft and Kamloops populations. Host weight (age) at oviposition is known to affect adult parasite weight (Chapter 4). The observed parasite weight differences between the two populations could be the result of differences in aphid population age-structure between the two locations at the time of parasitization. It could also be the result of differences in abiotic parameters (e.g. temperature, precipitation) and biotic parameters (predators, secondary parasites) between the two locations.

Using the relationships between host age at oviposition and adult parasite weight (Chapter 4), it is possible to examine the effect of host age structure on the distribution of adult parasite weights. Aphids ranging in age from 0.5 to 6.0 days (in 0.5 day increments) were used as hosts in Chapter 4. The

'laboratory' histograms given in Figs. 7 and 8 were obtained by pooling the adult parasite weights obtained from each age of host aphid in Chapter 4. In the tabulation of these histograms, the frequencies of each age class from each age of host aphid were weighted by the reciprocal of the sample size of their host age group. This resulted in each host age group contributing an equal amount (i.e. one-twelfth of the total) towards the total histogram. Thus, the 'laboratory' histograms in Figs. 7 and 8 represent the expected adult parasite weight distributions when all ages of host aphid (from 0.5 to 6.0 days) are parasitized with equal frequencies. Since adult parasite weight is a function of host age (weight) at oviposition (Chapter 4), the shape of the parasite weight distribution (in particular the third and fourth moment statistics) will be a function of the age structure of the hosts at the time of oviposition.

In the field, the situation is much more complex. It can be assumed that aphid populations in mature alfalfa fields will contain individuals of all ages, and, further, that A. smithi attacks all ages of host aphid in the field (Campbell 1973). Thus, parasite weight distributions from field data should contain individuals having developed in every age of host

Figure 7. Male Aphidius smithi adult weights sampled from field populations in Ashcroft and Kamloops and obtained from known age hosts in the laboratory.

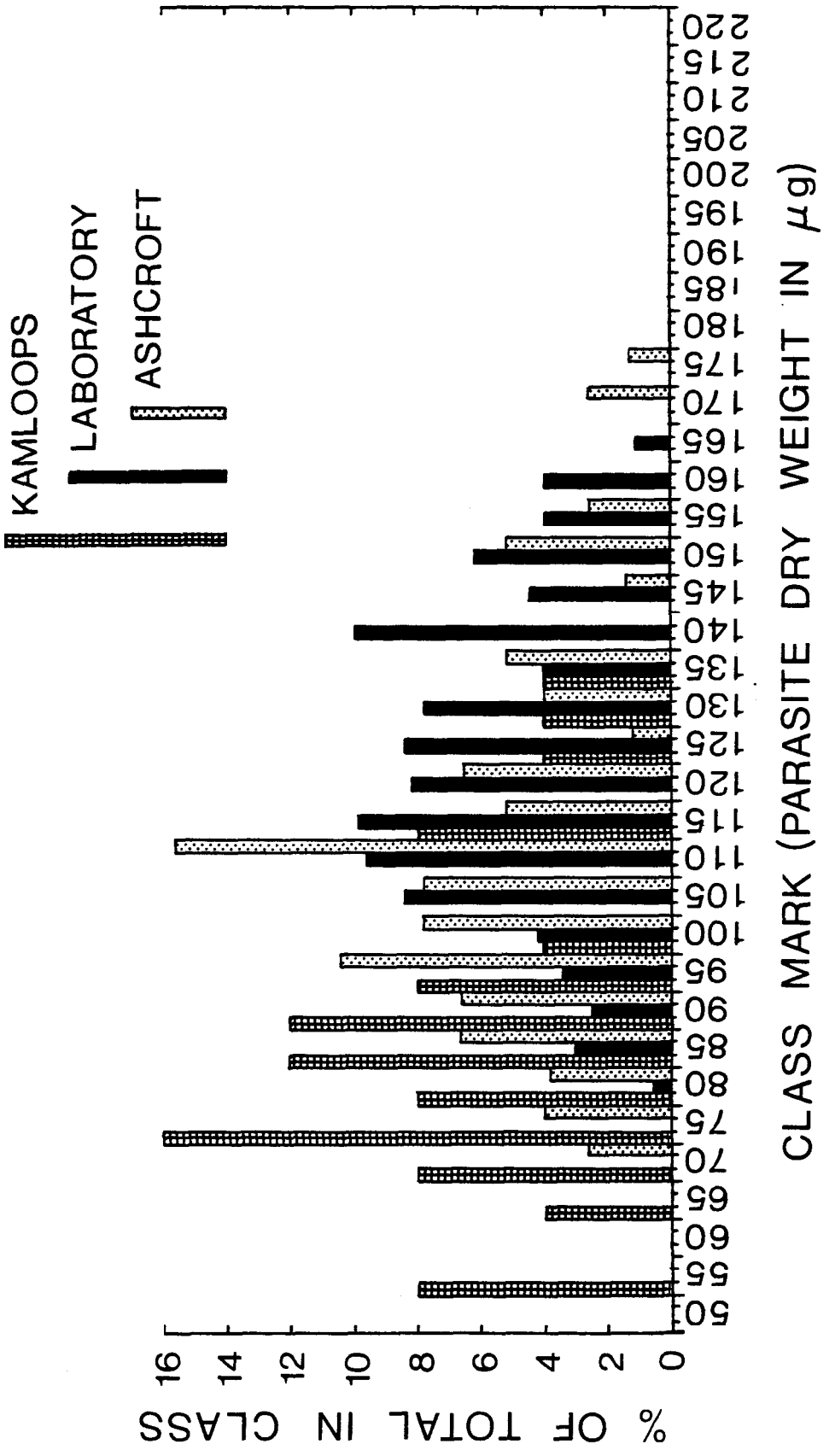
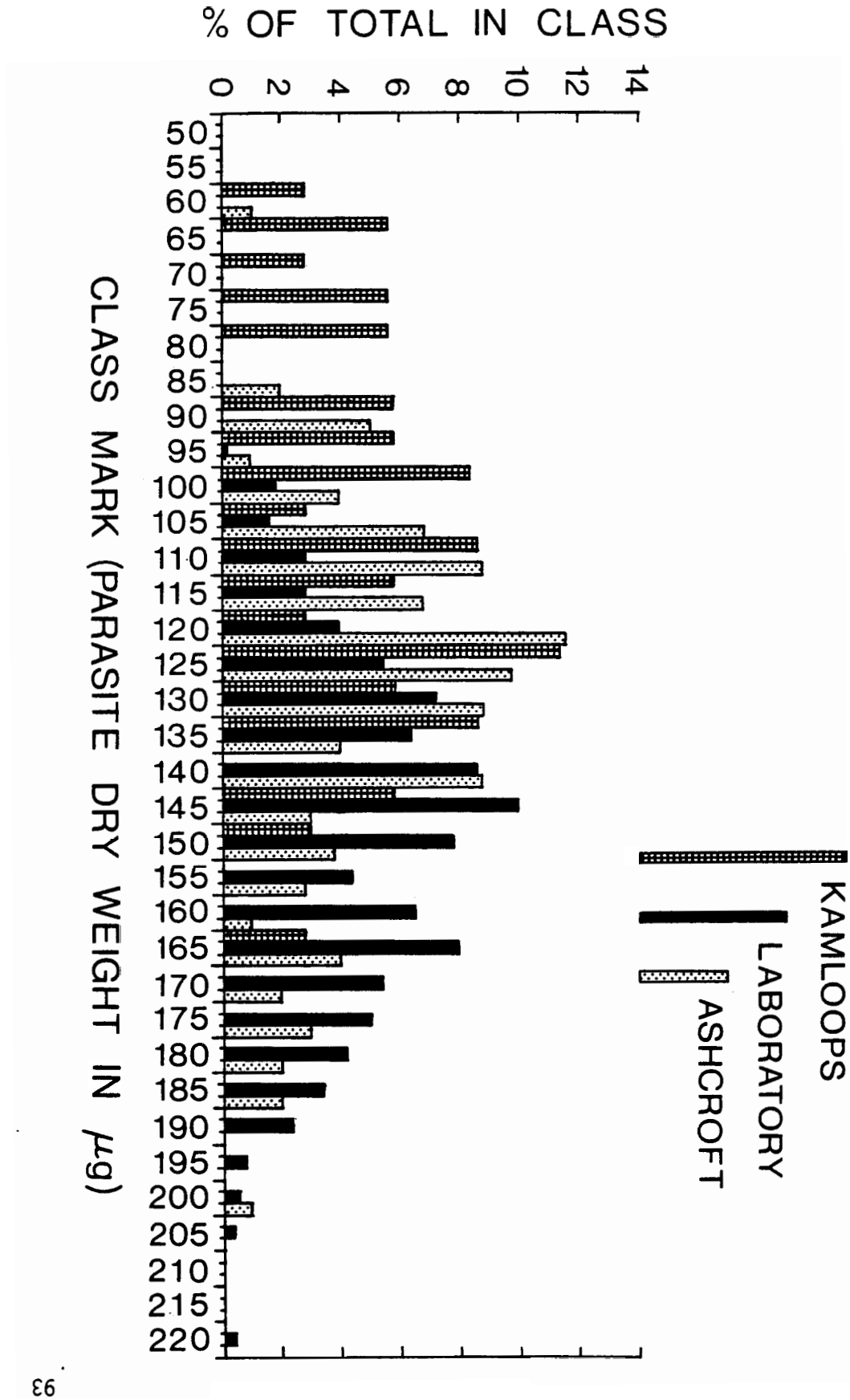

KAMLOOPS
LABORATORY
ASHCROFT
% OF TOTAL IN CLASS
0
2
4
6
8
10
12
14
16
CLASS MARK (PARASITE DRY WEIGHT IN μg)
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140
145
150
155
160
165
170
175
180
185
190
195
200
205
210
215
220

Figure 8. Female Aphidius smithi adult weights sampled from field populations in Ashcroft and Kamloops and obtained from known age hosts in the laboratory.


% OF TOTAL IN CLASS
0
2
4
6
8
10
12
14
CLASS MARK (PARASITE DRY WEIGHT IN μg)
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140
145
150
155
160
165
170
175
180
185
190
195
200
205
210
215
220
KAMLOOPS
LABORATORY
ASHCROFT

aphid. If host age at oviposition is the principal cause of parasite weight variation in the field (and this is by no means certain), then the shape of the parasite weights distributions obtained from the field should reflect the host aphid age structure at the time of oviposition. Environmental differences between locations should affect all individuals equally, and thus shift the entire distribution towards higher cr lower parasite weights, but the shape of the distribution should remain unchanged.

The weight distributions of the adult parasites from the Ashcroft and Kamloops field populations are also presented in Figs. 7 and 8. Comparing the general shapes of the Ashcroft and 'laboratory' distributicns, it appears as if the Ashcroft distribution contains more individuals in the lower weight classes than the 'laboratory' distribution. This suggests that in the field lighter (younger) aphids more frequently act as parasite hosts than heavier aphids. This could be the result of younger aphids having a higher frequency in the unparasitized aphid population, or it could be the result of preferential host selection by the adult parasite (Fox et al. 1967; Mackauer 1973; Wiackowski 1962).

The sample sizes in the Kamloops distributions are

too small (25 males and 35 females) to comment on the shapes of the distributions, but it is notable that the entire distributions appear to be shifted towards lighter parasite weights (Figs. 7 and 8). This suggests that the quality of the environment is lower (poor host quality, high or low temperature) in Kamloops than in Ashcroft or the laboratory.

The above examination of weight variation in field populations is by necessity incomplete, since an in-depth study would have required many years of research. Other causes of parasite weight variation in the field (such as age-specific mortality) need to be examined. Although incomplete, the above study does indicate that measuring weight variation may be a useful approach to the study of A. smithi population ecology.

5.4 SUMMARY

There is significant phenotypic variation in adult A. smithi weight between Ashcroft and Kamloops field populations. This between-population variation was not present in F_1 males reared in a constant laboratory environment, suggesting that the observed geographic variation in the field populations is caused by environmental differences between Ashcroft and Kamloops. A comparison of the frequency distributions of the weights in the field populations with those obtained in the laboratory suggested that lighter (younger) aphids are more likely to be parasite hosts in the field than older aphids, and that environmental parameters other than host age structure reduced the adult parasite weights in the Kamloops population.

CHAPTER 6

ARTIFICIAL SELECTION FOR HEAVY AND LIGHT POPULATIONS OF APHIDIUS SMITHI

6.1 INTRODUCTION

Artificial selection has been used for decades, principally by animal and plant breeders, to modify quantitative traits in animal and plant populations. The success of these artificial selection programs provides the strongest evidence for the presence of widespread variation in the genes affecting traits of adaptive significance (Lewontin 1974).

The level and rate of response to artificial selection can be improved by efficiently choosing the parents of each succeeding generation and by maximizing the heritability of the trait being selected, either by increasing the genetic component, or by decreasing the environmental component, of the variation in the trait (Falconer 1960). The genetic component of the variation

in the trait can be increased by synthesizing the base population from individuals having diverse genetic backgrounds and by maintaining this genetic diversity through the minimization of inbreeding. The environmental component of the variation in the trait can be reduced by maintaining constant those environmental parameters that most affect the trait.

The choice of parents for each succeeding generation in a selection program will depend upon the selection method. The selection method that should always yield the most rapid response to selection is combined selection (Falconer 1960). Combined selection utilizes information on both the family mean and the within-family variance to choose the parents for the next generation. However, combined selection is usually not used, because the small increase in expected response does not compensate for the increased complexity in the choice of parents. The selection method, other than combined selection, that should yield the most rapid response to selection can be determined by calculating the intraclass correlation (t). This statistic is the variance component among families as a proportion of the sum of the variance component among families and the variance within families. When t is large, each family experiences a

unique common environment, and the best method of selection is within-family selection. When t is small, the families are all experiencing a similar environment and the heritability of the trait is low. The best selection method in such cases is family selection. When t is intermediate, the best selection method is individual selection. Falconer (1960) graphically compares the expected response from the above three methods of selection relative to combined selection as a function of t.

Artificial selection programs for increased or decreased size or weight have been successful with many organisms (Enfield et al. 1966; Falconer 1953; Katz and Young 1975; Robertson and Reeve 1952; Tantawy and El-Helw 1966). The heritability of size or weight in previous selection programs has been increased by rearing the organisms involved on well-defined diets. No attempts have previously been made to artificially select for size or weight in an organism, such as A. smithi, that must develop within the living tissues of another organism.

In this chapter, the response to artificial selection for increased and decreased A. smithi mummy weight is examined. Knowledge gained on the changes in

individual weight over time (Chapter 3) and on the effect of host weight on parasite weight (Chapter 4) is used to reduce the environmental component of weight variation.

There are many reasons for conducting this selection experiment. It demonstrates that it is technically possible to conduct an individual selection program for weight in a system as complex as the A. smithi-pea aphid-broad bean system. It is possible to improve biological control agents by selectively breeding for desirable attributes (Mackauer 1976). Since many biological control agents are parasitic insects, information gained from weight selection in A. smithi will be useful for designing other selection programs on parasitic insects. If successful, the experiment would have produced two populations that genetically differ in weight. These populations could have been analyzed for correlated responses to selection, and, by rearing them on a series of host ages, for genotype - environment interaction.

6.2 MATERIALS AND METHODS

The A. smithi parasites that formed the base population for the selection experiment were taken from a stock colony having the following origin: In July of 1976, a large proportion of the A. smithi parasites collected from fields in Ashcroft and Kamloops (Chapter 5) were used to establish 'Ashcroft' and 'Kamloops' laboratory colonies. At that time, there was also an A. smithi laboratory stock colony, which had originated from parasites collected in the Kamloops region, and had been maintained in the laboratory for several years. In order to increase the genetic variability in the laboratory stock colony, a new laboratory stock colony was formed in September 1976 by combining approximately 200 individuals from each of the old laboratory stock colony, the 'Ashcroft' laboratory colony, and the 'Kamloops' laboratory colony. The population size of this new stock colony was kept at a minimum of 300 individuals to minimize any loss of genetic variability. In April of 1977, the new stock colony was used as a source of parasites for the base population of the selection experiment.

In order to reduce the environmental component, and thus increase the genetic component, of the weight variance, it was essential to begin the selection from

a base population that had been reared on constant age host aphids, because the age of the host aphid is known to affect parasite weight (Chapter 4). The base population for the selection was obtained as follows: Several hundred mummies were removed from the stock colony and placed into gelatin capsules. Twenty-nine males and 29 females were randomly chosen from the parasites emerging from these mummies and were mated. In each of 5 ovipositional sessions, 60 to 105 potentially parasitized aphids were obtained by allowing each of 4 to 7 mated female parasites to oviposit into five 48 $\pm$ 2.5 hour old (second instar) host aphids approximately every 20 minutes for approximately 1 hour. In order to determine aphid weight at oviposition, 2 'control' aphids were randomly sampled from each of the 9 plastic cages that were used to rear the host aphids for each ovipositional session. After oviposition, the potentially parasitized aphids were placed on plants in rearing cages, one cage for all the aphids (N=15) attacked by each female parasite, and allowed to develop. Thus, each cage contained full siblings. The mummies containing the developing parasites were removed from the plant 10 days after oviposition.

The relative individual parasite weights in the

base population were determined by weighing the vacated mummies. For this experiment, mummy weight was considered to be the most feasible of the three weight variates discussed in Chapter 3. Pupa weight 9 days after oviposition was not used since the environment experienced by the parasites in each generation could easily vary during this 5 month experiment. Such variations would alter the physiological age, and thus the weight (Chapter 3), of the parasites at 9 days after oviposition, making it difficult to compare the weights from generation to generation. Moreover, mummy weight is easier to measure than pupa weight 9 days after oviposition. Adult dry weight could not be used since live adults were needed as parents for subsequent generations. All mummy weights were adjusted for missing aphid appendages using the adjustment factors calculated in Chapter 4.

Analysis of the mummy weights in the base population (see Results and Discussion) revealed that individual selection was the selection method that would yield the most rapid response. Thus, the parents of generation 1 of the heavy and light populations were selected from the base population according to the following procedure: All the parasites in the base population were ranked according to mummy weight. The

21 male and 21 female parasites having the heaviest mummy weight were selected as parents for generation 1 of the 'heavy' population, except when more than 3 individuals of one sex came from the same cage (i.e. were full sibs, since each cage contained one family). In such cases the fourth (lightest) mummy was rejected, and the parasite having the next heaviest mummy in the rank was selected. This restriction reduced the level of inbreeding accompanying the selection. The 21 males and 21 females so selected were mated at random and used as parents for generation 1 of the 'heavy' population. The 21 males and 21 females used as parents for generation 1 of the 'light' population were selected in a similar manner from the light end of the ranked mummy weights of the base population.

The above selection procedure was used for all subsequent generations, except that the parents for the 'heavy' population were selected from the previous generation's 'heavy' population, and the parents for the 'light' population were selected from the previous generation's 'light' population (i.e. both selected populations remained genetically distinct after the base population).

After selecting and mating the parents, each generation of each population was obtained as follows:

In each of three ovipositional sessions, 105 potentially parasitized aphids were obtained by allowing each of 7 mated female parasites to oviposit into five 48 ± 2.5 hour-old (second instar) host aphids approximately every 20 minutes for approximately 1 hour. In order to determine aphid weight at oviposition, 2 'control' aphids were randomly sampled from each of the 8 cages that were used to rear the host aphids for each ovipositional session. After oviposition, the potentially parasitized aphids were placed in rearing cages, one cage for all the aphids (N=15) attacked by each female parasite, and allowed to develop. The mummies containing the developing parasites were removed from the plant 10 to 11 days after oviposition.

The weights of the mummies from each ovipositional session in each generation of each population were adjusted to compensate for deviations in the control aphid dry weight from 59.2μg (see Results and Discussion).

The intensity of selection (Falconer 1960) was calculated separately for each sex in each generation

as: $i = S/\sigma_p$

where:

S = the selection differential

= the mean mummy weight of the individuals selected as parents expressed as a deviation from the population mean

σ_p = the standard deviation of mummy weights

The mummy weights of the heavy and light populations were compared for each sex in each generation using a two level mixed model nested ANOVA. The model used was:

$$\overline{AB}_{ij} = \mu + \alpha_i + \beta_{j(i)} + \bar{\varepsilon}_{ij}$$

where:

$\overline{AB}_{ij}$ = the mean weight in cell ij

μ = the parametric grand mean

α_i = the fixed treatment effect due to selection

$\beta_{j(i)}$ = the nested random variance component due to parents and cages

$\bar{\varepsilon}_{ij}$ = the mean experimental error in cell ij

6.3 RESULTS AND DISCUSSION

Table 15 presents the single classification (into families) ANOVAs that yielded the variance estimates used to calculate the intraclass correlations of the mummy weights in the base population. The intraclass correlation was 0.325 for the males and 0.426 for the females (Table 15). The highly significant among-family variance component is a consequence of the common environment and the common genes shared by the individuals within each family. Because of the intermediate intraclass correlations in both males and females, individual selection was the method chosen for the artificial selection of heavy and light populations.

Table 16 presents statistics on the dry weight of the host aphids sampled at the time of oviposition from each population in each generation. In 4 of the 9 selection generations, there was a significant difference between the weights of the aphids used as hosts for the light population and those used as hosts for the heavy population (Table 16). Moreover, there was a significant ($p<0.001$) difference in the host aphid weight between generations. This host aphid weight variation could be caused by differences in temperature, host plant quality, aphid stock colony

Table 15 A single classification (into families) ANOVA of *Aphidius smithi* base population vacated mummy weight (μg). A) Males. B) Females.

A)

Source of variation	DF	SS	MS	F
Among families	28	21486	767.36	3.81***
Within families	141	28413	201.51	

n=5.82; t=0.325

B)

Source of variation	DF	SS	MS	F
Among families	27	20485	758.70	5.16***
Within families	130	19128	147.14	

n=5.60; t=0.426

*** $p<0.001$

note: $s^2 = MS_W$

$s_A^2 = (MS_A - MS_W)/n$

$t = s_A^2/(s^2 + s_A^2)$

Table 16 Statistics on the dry weight (µg) of the pea aphid controls sampled at the time of oviposition.

Gen.	Pop.	Mean	N	Standard deviation	
Base	-	61.14	80	8.31	
1	Light	69.96	48	8.62	n.s.
1	Heavy	69.69	48	7.46	
2	Light	60.29	48	7.94	n.s.
2	Heavy	59.96	48	7.86	
3	Light	66.81	48	7.97	n.s.
3	Heavy	65.69	48	7.91	
4	Light	72.23	48	9.92	***
4	Heavy	65.17	48	6.33	
5	Light	59.58	48	7.39	**
5	Heavy	54.92	48	8.42	
6	Light	69.69	48	7.60	**
6	Heavy	64.98	48	8.10	
7	Light	63.81	48	7.97	n.s.
7	Heavy	62.60	48	7.65	
8	Light	57.54	48	9.27	***
8	Heavy	51.54	48	7.26	
9	Light	59.02	48	6.93	n.s.
9	Heavy	59.12	48	8.79	

n.s. not significant; ** $p<0.01$; *** $p<0.001$

note: the asterisks show the results of an ANOVA comparing the controls from the heavy population with those from the light population

quality or aphid crowding, both within one generation and between generations.

Since host aphid weight is known to affect mummy weight (Chapter 4), it was necessary to adjust the weights of the mummies for differences in the weights of their host aphids. A relationship was needed between host aphid weight at oviposition and mummy weight for the range of host aphid weights encountered in the selection experiment. This relationship was determined by using ANOVA with regression to analyze the mummy weights, obtained in Chapter 4, from host aphids ranging in weight from 45.24g and 92.44g. The results of the analyses are presented in Table 17. In both males and females, the regression removed a highly significant component of the variance among host weight groups. The deviations from regression were not significant for either sex. The slope of the regression line is 0.490 for females and 0.645 for males. An adjustment factor was calculated for each sex in each ovipositional session by multiplying the slope of the regression line for that sex by the difference (including magnitude and sign) between 59.24g and the mean host aphid weight of the control aphids from that ovipositional session. The value of 59.24g was chosen as the standard since it was the aphid weight observed

Table 17 An analysis of variance of Aphidius smithi mummy weight (µg) regressed on host weight (µg) at oviposition for hosts between 45.2µg (1.5 days) and 92.4µg (3.0 days). A) Males. B) Females.

A)

Source of variation	DF	SS	MS	F	
Among host weight groups	3	32567	10856	38.88	***
Linear regression	1	32407	32407	404.37	***
Deviations from regression	2	160	80	0.29	n.s.
Error	273	76221	279		

B)

Source of variation	DF	SS	MS	F	
Among host weight groups	3	9844	3281	7.81	***
Linear regression	1	9680	9680	118.40	***
Deviations from regression	2	164	82	0.19	n.s.
Error	116	48712	420		

n.s. not significant; *** $p<0.001$

at 48 hours of age in Chapter 4 (Fig. 6). Each mummy weight in the selection experiment was adjusted for its host aphid weight by adding the appropriate adjustment factor to the measured mummy weight. All subsequent analyses were performed on adjusted mummy weights.

The results of the selection program are shown in Fig. 9. The selection differentials (S) and the intensities of selection (i) are given in Table 18.

From generation 3 to generation 9 inclusive, the light population consistently had lighter mean mummy weights than the heavy population for both males and females (Fig. 9). The relationship between the mean mummy weights of the two populations in generations 1 to 9 inclusive was analyzed statistically using the non-parametric Wilcoxon matched-pairs signed-ranks test (Siegel 1966). The null hypothesis was that in each generation there was no difference between the mean mummy weights in the two populations. The alternative hypothesis was that the mean mummy weight in the heavy population was greater than that in the light population. The results demonstrated that, in both sexes, the probability is less than 0.025 (males: T=5.5; females: T=3.0) that the mean mummy weights in the heavy and light populations are identical.

Figure 9. The response to artificial selection for increased and decreased mummy weight in Aphidius smithi. A) Males B) Females (● selected for increased weight; ○ selected for decreased weight).

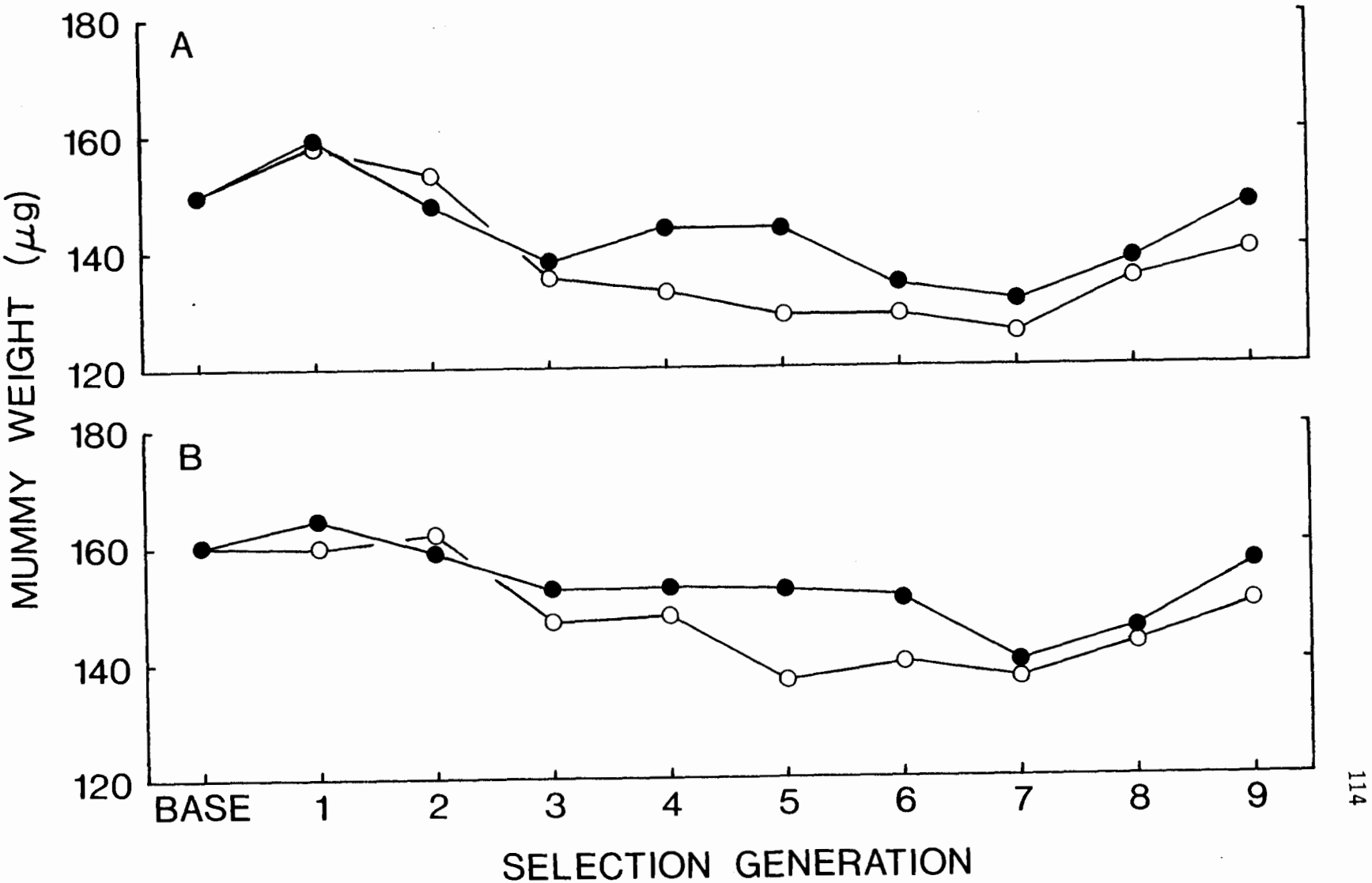

A
B
180
160
140
120
MUMMY WEIGHT (μg)
BASE
1
2
3
4
5
6
7
8
9
SELECTION GENERATION

Table 18 Selection differentials (S) and intensities of selection (i) in the Aphidius smithi weight selection program.

Generation	Population	Males		Females	
		S[a]	i	S[a]	i
Base to 1	Light	29.1	1.69	22.3	1.42
Base to 1	Heavy	26.4	1.53	23.5	1.49
1 to 2	Light	20.1	1.28	20.8	1.33
1 to 2	Heavy	20.4	1.36	18.9	1.31
2 to 3	Light	19.7	1.46	19.6	1.22
2 to 3	Heavy	21.8	1.19	17.8	1.09
3 to 4	Light	20.9	1.26	23.0	1.34
3 to 4	Heavy	22.0	1.41	20.9	1.37
4 to 5	Light	21.4	1.30	16.8	1.22
4 to 5	Heavy	19.1	1.26	20.0	1.14
5 to 6	Light	16.7	1.02	19.3	1.22
5 to 6	Heavy	18.4	1.20	19.0	1.41
6 to 7	Light	17.2	1.16	16.0	1.11
6 to 7	Heavy	20.4	1.45	17.4	1.37
7 to 8	Light	15.2	1.00	20.7	1.10
7 to 8	Heavy	20.7	1.18	25.5	1.44
8 to 9	Light	19.3	1.37	13.1	0.93
8 to 9	Heavy	23.0	1.51	23.0	1.28

a) in µg

Thus, artificial selection did result in a divergence in mummy weight between the two populations.

The extent of the divergence in mummy weights between the two populations was examined at each generation using nested ANOVA. The results for each generation of each sex are given in Table 19. In generation 5 of the females, and in generations 4, 5 and 9 in the males, the mummy weights of the heavy populations were statistically heavier than those of the light population. However, the ANOVA results do not demonstrate a consistent separation of the mummy weights of the two populations in the later generations of either sex. The separation in generation 5 should have remained in the succeeding generations, particularly since the parents of these generations were still being selected for mummy weight. Since the divergence between the two populations is not great, there is a high probability of Type II error with these analyses of variance. Thus, although the relative magnitudes of the mean mummy weights between the populations over generations indicates separation, the separation is insufficient to reveal significant differences between the individual mummy weights of the two populations in every generation.

It is difficult to determine how the selected

Table 19 Summary of two-level nested ANOVA's of *Aphidius smithi* mummy weight (*ug*) at each generation of the selection program.

Gen.	Sex	Variance among families DF	F	Variance among populations DF	F
1	Male	40/240	3.51 ***	1/36.3	0.08 n.s.
1	Female	37/187	3.73 ***	1/33.3	1.81 n.s.
2	Male	40/267	5.94 ***	1/38.3	1.64 n.s.
2	Female	37/193	2.70 ***	1/33.6	0.85 n.s.
3	Male	40/219	7.08 ***	1/38.4	0.20 n.s
3	Female	40/206	5.11 ***	1/37.4	2.07 **
4	Male	40/207	3.70 ***	1/35.5	11.72 n.s.
4	Female	38/220	3.69 ***	1/34.0	1.87 ***
5	Male	40/199	5.43 ***	1/37.8	15.29 ***
5	Female	39/227	5.09 ***	1/37.0	21.32 n.s.
6	Male	40/197	4.39 ***	1/37.5	2.49 n.s.
6	Female	37/201	4.70 ***	1/34.1	2.96 n.s.
7	Male	40/206	6.37 ***	1/37.9	1.28 n.s.
7	Female	40/205	4.87 ***	1/36.7	0.09 n.s.
8	Male	37/276	6.25 ***	1/36.0	0.68 n.s.
8	Female	34/134	2.65 ***	1/30.8	0.31 *
9	Male	38/168	4.17 ***	1/34.1	4.19 n.s.
9	Female	39/263	7.40	1/37.3	1.41

n.s. not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$

populations have changed relative to the base population, since an unselected control population was not weighed in each generation along with the two selected populations. The occurrence of parallel changes in mean mummy weight of the heavy and light populations, particularly in generations 6 to 9 (Fig. 9), suggests that the mummy weights were being affected by environmental parameters common to both populations within a generation, but not constant over all generations.

The response to artificial selection for weight or size in other organisms has been much greater than that observed with A. smithi in this study. The divergence between the two populations after 9 generations of selection (7.8μg in males; 5.7μg in females (Fig.9)) is only 5.2% (males) and 3.6% (females) of the weight in the base population. When selecting for wing length in D. melanogaster, Tantawy and El-Helw (1966) produced a divergence between large and small lines of over 10% of the control wing length after 10 generations. When selecting for 6-week weight in mice, Falconer (1953) produced a divergence between heavy and light lines of approximately 45% of the base population weight after 11 generations. Enfield et al. (1966) produced a 32% increase in Tribolium castaneum pupa weight after 12

generations. Katz and Young (1975) produced a 25% increase in D. melanogaster adult body weight after 18 generations. There are several possible reasons for the lower response to weight selection in A. smithi.

Most other selection programs involving size or weight have used organisms that feed on a non-living, well-defined diet. Such diets will reduce the environmental component of weight variance and thus increase the heritability and the expected response to selection. A. smithi is a parasite of an insect which in turn feeds on living plant tissue. The inherent variation in the food chain will greatly increase the environmental component of A. smithi weight variation.

The low response to selection observed in this experiment could also have been a consequence of the genetic structure of the base population. The base population was synthesized from field populations that, because of their origin (Chapter 5), are potentially low in genetic variance. Parent-offspring regression of the laboratory-reared F_1 males on their field collected female parents (data from Chapter 5) yielded a slope of 0.073 ± 0.047. Thus, the heritability of weight in the base population may have been very low.

It is not known what effect the A. smithi haplodiploid genetic system may have had on the

selection results. The amount of variation maintained by epistasis is expected to be smaller in a haplodiploid genetic system (Hartl 1971). Reduced electrophoretic variation has been observed in many haplodiploid organisms (Lester 1975; Mestriner 1969; Mestriner and Contel 1972; Snyder 1974). Given the same parameters in the base population, a haplodiploid population should evolve up to one-third faster than a corresponding diploid-diploid population (Hartl 1971). Selection programs in other haplodiploid organisms have been successful (De Bach 1958; Rothenbuhler et al. 1968; White et al. 1970).

Perhaps the most significant factor that restricted the divergence between the two populations is the complex association between the parasite and its host. Successful selection for increased or decreased weight requires modification of the parasite while maintaining a constant host. This constant host will also place restrictions on parasite growth by limiting the quantity of food available to the developing parasite larva. The fact that artificial selection did not result in an increase in mummy weight over that of the base population (Fig. 9) suggests that it is easier to disrupt the host-parasite association (that is, select for decreased mummy weight), than it is to

improve the association (that is, select for increased mummy weight). A slower response to selection for larger than for smaller size has been observed in Drosophila melanogaster (Robertson and Reeve 1952) and mice (Falconer 1953).

Further experiments were not conducted on the heavy and light populations at the end of the selection program due to the low divergence between the two populations.

6.4 SUMMARY

Artificial selection for populations of A. smithi having increased and decreased mummy weight resulted in the light population consistently having a lower mean mummy weight than the heavy population from generation 3 to generation 9. When examined over generations, the difference in mean mummy weight between the populations indicated that the heavy population was significantly heavier than the light population. When each generation was examined separately, significant differences between the individual mummy weights in the two populations were only observed in generation 5 of the females and generations 4, 5 and 9 of the males. Artificial selection did not result in an increase in mummy weight over that of the base population.

CHAPTER 7

CONCLUDING REMARKS

With weight in A. smithi, variation is the rule rather than the exception. It is the presence of this variation, and its dependence upon other parameters of interest to the population biologist, that makes the study of weight a worthwhile venture. As well as being the expression of the genotype, the weight of an individual is an expression of the history of an individual, in essence, a cumulative bioassay of the environment in which the individual developed. Enumerating the individuals in a population only touches the surface of the available information. There is a need to go beyond this typological approach; individuals are different, and this difference is important.

The problem with variation is that it is difficult to interpret; the individual causes of the variation

must be understood. After minimizing the within-individual component of weight variation, it was found that much of the variation in parasite weight is caused by the limits placed on parasite growth by the quantity of food available in the host. Measurable geographic variation in parasite weight was found to be caused by extrinsic environmental differences between the two locations; host age variation is probably a major cause of the intrinsic parasite variation at the two locations. The restrictions placed on parasite growth by the host also limited the potential divergence between artificially selected heavy and light populations. Variation in host weight is an inseparable part of variation in parasite weight. It is hoped that this thesis will contribute towards the understanding of both parasite weight variation and the intricate association between a parasite and its host.

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