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Selected biological characteristics of Brachymeria ovata reared on live and freezer-stored pupae of the cabbage looper, Trichoplusia ni

Russell Louis Smith

To the Graduate Council:

I am submitting herewith a thesis written by Russell Louis Smith entitled "Selected biological characteristics of Brachymeria ovata reared on live and freezer-stored pupae of the cabbage looper, Trichoplusia ni." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Jerome F. Grant, Major Professor

We have read this thesis and recommend its acceptance:

Paris L. Lambdin, M. L. Pan

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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SELECTED BIOLOGICAL CHARACTERISTICS OF *Brachymeria ovata* REARED ON LIVE AND FREEZER-STORED PUPAE OF THE CABBAGE LOOPER, *Trichoplusia ni*

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Russell Louis Smith

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DEDICATION

This thesis is dedicated to my parents and the many professors who have encouraged my study of insects.

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Voucher specimens of *Brachymeria ovata* and *Trichoplusia ni* were deposited in the University of Tennessee, Department of Entomology and Plant Pathology Insect Museum.

ABSTRACT

Parasitoids can be an important part of a biological control program. The use of freezer-stored pupae has been suggested as a means to rear large numbers of parasitoids for release in biological control programs (Grant and Shepard 1987). *Brachymeria ovata* (Say) (Hymenoptera: Chalcididae) has been reared successfully from freezer-stored pupae of several noctuid species of lepidoptera (Grant and Shepard 1987); however, it was not known if the use of freezer-stored pupae as hosts of *B. ovata* had any affect on the biological characteristics of this parasitoid.

A colony of *B. ovata* was maintained, and all experiments were conducted, utilizing the pupae of the cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae). Experiments were conducted at 27±2°C and 50 to 65%RH.

The mating ability of individuals reared from freezer-stored pupae was not significantly impacted; however, a slight decrease in successful matings was observed when males were reared from freezer-stored pupae. The mean preovipositional period increased when individuals were reared from freezer-stored pupae. *B. ovata* generally parasitized pupae during the hours of light. The greatest number of parasitoids were produced during the first four-hour period of light and the number of progeny produced decreased throughout the day.

Unmated female *B. ovata* individuals not exposed to hosts generally lived longer (114.0 and 135.8 days, respectively, when reared from live and freezer-stored pupae) than unmated males (approximately 100 days), and those females reared from freezer-stored pupae lived significantly longer than females reared from live pupae. Paired adults lived for shorter periods than unmated *B. ovata*. Longevity of paired females exposed to live pupae was significantly shorter (approximately 15 days) than the longevity of males or females not exposed to live or freezer-stored pupae (approximately 60 to 86 days, respectively).

Fecundity of *B. ovata* increased when females were exposed to freezer-stored pupae. Those females reared on and exposed to freezer-stored pupae produced the greatest number of eggs ($\overline{x}$=422.2). Females exposed to live pupae lived one fourth to one third as long when reared from live or freezer-stored pupae, respectively, and produced an average of 91.3 and 137.2 eggs, respectively.

Progeny production was significantly decreased when females were exposed to freezer-stored pupae. Females produced an average of 83.3 and 79.1 progeny when reared from live or freezer-stored pupae, respectively, and exposed to live pupae during their entire lifetime. Females exposed to freezer-stored pupae produced significantly fewer progeny ($\overline{x}$=31.9 and 37.3, respectively, when reared from live or freezer-stored pupae) during the first 14 days of oviposition. As the fecundity of individuals exposed to freezer-stored

pupae is greater than that of ones exposed to live pupae, the potential exists for greater progeny production from females exposed to freezer-stored pupae.

The acceptability of freezer-stored pupae was significantly lower than that of live pupae in terms of the percentage of pupae containing eggs (88.2% of live pupae and 71.4% of freezer-stored pupae) but the average number of eggs per pupa was not significantly different. The suitability of live and freezer-stored pupae in terms of the percentage of pupae supporting development of *B. ovata* to adulthood was significantly higher for live pupae (85.5%) than for freezer-stored pupae (23.0%) Higher levels of suitability were observed in other experiments utilizing freezer-stored pupae.

The suitability of different age pupae, at time of freezing, and of live pupae also, was determined to be from two to five days after pupation. Many individuals reared on older pupae were smaller than those reared on pupae two to five days old. The production of small adults was probably a result of having less food available to the larva. The development of immature stages of *B. ovata* in freezer-stored pupae was lengthened from two to five days in the larval stage and about one day in the pupal stage.

Overall freezer-stored pupae did not detrimentally impact upon the biological characteristics of *B. ovata*. With increased suitability of freezer-stored pupae as observed in some experiments conducted in this study as well as others (Grant and Shepard 1987), it should be economically advantageous to use freezer-stored pupae as a host for *B. ovata*.

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INTRODUCTION

Parasitic insects are both ecologically and economically important. Askew (1971) estimated that one tenth of all animal species are parasitic insects, ranging from ectoparasites of mammals, such as a flea, to tertiary hyperparasites, that is, a parasite that attacks the parasite of another parasite. Price (1975) considered leafhoppers to be plant parasites comparable to body lice, and a seed beetle to be a parasitoid of a plant as a tachinid may be a parasitoid of a caterpillar. The term "parasitoid" has been considered by some authors to be vague and taxonomically restricted (Eggleton and Gaston 1990). Askew (1971) referred to parasitoids as protelean parasites. Parasitoid is commonly used only in entomological literature to describe an insect species whose larvae develop on or within an insect host's body, ultimately killing the host; adult females are free living, usually feeding on nectar and pollen (Eggleton and Gaston 1990). The term parasitoid will be used in this manner throughout this thesis.

As discussed previously, about one half of the animals on earth are parasitic insects as estimated by Price (1975). The existence of many parasitic animals makes it possible, in theory, to control many plant and animal pest species in biological control programs. Applied biological control, as discussed

here, is the use of a living organism to control another organism considered to be a pest species (Metcalf and Metcalf 1982).

With concerns about pollution and food contamination caused by insecticides, the use of biological control, and its incorporation into integrated pest management programs, will become increasingly important. As urban areas encroach further into agricultural areas, homeowners will demand a reduction in the use of chemical pest controls. In some cases, biological control provides an excellent opportunity to reduce the amount of pesticides needed to protect crops from pests, and parasitoids can be an important part of a biological control program. According to the Commonwealth Agriculture Bureaux (CAB) International Institute for Biological Control, at least 860 successful establishments of 393 species of parasitoids against 274 insect pests in 99 countries have occurred (Waage and Greathead 1986). Of these establishments, 216 achieved complete or satisfactory pest control, while 52 other species achieved a useful reduction in the pest population. Some of the establishments of parasitoids that were rated as successful also included introduced predators.

The use of parasitoids in biological control programs, whether for inundative or inoculative releases, usually requires that many parasitoids be released simultaneously or periodically. Ideally, one could rear parasitoids on an artificial diet in the laboratory to eliminate the need for maintaining a host colony. Research is underway to evaluate the use of artificial diets for rearing parasitoids, but the application of this procedure is currently feasible for only a

few parasitoid species (Nettles, W. C., Jr., personal communication). Rearing parasitoids on artificial diet may cause problems, such as the development of smaller adults which may result in lower fecundity, prolonged development, and a reduced percentage of individuals surviving to adulthood. The elimination of natural hosts also requires the use of an ovipositional stimulant, which, in some instances may be incorporated into the artificial diet (Arthur et al. 1972).

The use of freezer-stored pupae for rearing large numbers of parasitoids for release in the field or use in the laboratory has been suggested by Grant and Shepard (1987). The use of freezer-stored pupae does not eliminate the need to maintain a host colony; however, the colony would not necessarily need to be continually maintained at a high level. Laboratories and insectaries that produce an excess number of pupae could freeze and ship pupae to other laboratories that rear parasitoids. This procedure may eliminate the need to maintain a host colony on the premises of the parasitoid rearing facility.

Parasitoids that have to be reared on a specific host could possibly be reared from freezer-stored hosts when supplies of live hosts are low due to laboratory rearing problems, when the host can not be reared in the laboratory, or when the host may not be available year round. Those parasitoids, such as *Brachymeria ovata* (Say) (Hymenoptera: Chalcididae), and *Brachmeria intermedia* (Nees) (Hymenoptera: Chalcididae), that can be reared on a number of host species, could be reared on the type of host most easily obtained or best suited for freezer storage.

The placement of parasitized pupae in the field before emergence of adult parasitoids is a convenient method to release parasitoids. However, when live pupae are exposed to a parasitoid colony, the possibility exists that some hosts may not become parasitized. Thus, when the hosts are placed in the field, some moths may emerge from nonparasitized pupae. The use of freezer-stored pupae eliminates the potential to add to the pest population in the field. Another advantage of the use of freezer-stored pupae in laboratory rearing of parasitoid species is that no adult moths will emerge in the parasitoid colony. Freezer-stored, or freezer-killed, pupae also can be used to monitor parasitoid populations in the field, or augment parasitoid populations by providing additional hosts for resident parasitoids to parasitize when pest populations are low (Petersen 1986). These additional hosts may allow the parasitoid population to remain high when the pest population becomes low, thereby providing greater suppression than if the parasitoid population followed the pest population.

Freezer-stored hosts have been used in parasitoid research, as well as several biological control programs. Frozen pupae of the house fly, *Musca domestica* L. (Diptera: Muscidae), have been used in a study of biological control of flies in poultry houses (Pickens and Miller 1978). The placement of frozen house fly pupae periodically in poultry houses after the introduction of parasitoids maintained the fly population at a level comparable to that of a poultry house that received periodic releases of parasitoids. House fly

populations in both types of poultry houses were reduced by 22 to 44% compared to a control house where no parasitoids were released.

Freeze-killed hosts have been used to release parasitoids into the field for control of house flies and stable flies, *Stomoxys calcitrans* (L.) (Diptera: Muscidae). To control flies in livestock confinements, pupae parasitized by three species of pteromalid wasps were placed in and around fly breeding habitats on dairies. Nonparasitized freeze-killed pupae also were placed in these areas to provide an early build up of fly parasitoids. *Muscidifurax zaraptor* Kogen and Legner (Hymenoptera: Pteromalidae) parasitized freeze-killed pupae in the field, and provided large numbers of a second generation of parasitoids (Petersen 1986). Freeze-killed house fly pupae also may be useful for monitoring parasitoid activity in the field.

The effects of using freezer-stored house fly pupae as hosts of the pteromalid parasitoid *M. zaraptor* were evaluated by Petersen and Matthews (1984). They found that freezer-stored pupae at all stages of development were suitable hosts. This finding differed from the suitability of live pupae, as one-day-old pupae were not suitable hosts. They also reported that freezer-stored pupae remained acceptable for as long as nine days at 26°C after removal from the freezer.

The development of *B. ovata* was evaluated on freezer-stored pupae of seven noctuid species (Grant and Shepard 1987). They found that pupae stored at -20°C and thawed four to six hours prior to exposure to parasitoids were

suitable hosts. Pupae of the velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae), were acceptable as hosts to *B. ovata* after freezer storage for 256 days. Pupae held as many as eight days at 27°C following freezer storage were also acceptable hosts. Progeny reared from freezer stored pupae were able to mate and reproduce. Egg parasitoids, such as *Ooencyrtus ennomophagus* Yoshimoto (Hymenoptera: Encyrtidae), have been reared on host eggs stored at -10°C for one month with no reduction in the percentage of parasitoids emerging as compared to fresh eggs (Drooz and Solomon 1980).

Ovipositional and olfactometer studies also have been conducted with *B. ovata* and freezer-stored pupae (Grant and Noblet 1991). They reported that *B. ovata* females preferentially oviposit in live pupae when presented with a choice between live and freezer-stored pupae. In no-choice tests, more than twice as many parasitoids emerged from live pupae as they did from freezer-stored pupae. When presented with a freezer-stored pupa and a blank cell in an olfactometer, the parasitoid chose the cell with the freezer-stored pupa, suggesting that freezer-stored pupae are recognized as hosts by *B. ovata* females.

Kairomones play an important role in host recognition by parasitoids (Askew 1971). Kairomones extracted from pupae of the gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae), using a hexane wash, were attractive to *B. intermedia* a pupal parasitoid of the gypsy moth. However, these same kairomones were not attractive to *Apanteles melanoscelus* (Ratzeburg) (Hymenoptera: Braconidae), a larval parasitoid. Similarly, kairomones extracted

from larvae were attractive to the larval parasitoid but not to *B. intermedia* (Leonard et al. 1975). Kairomones also play a role in the relative acceptance of different hosts for polyphagous parasitoids such as *B. ovata*. Studies utilizing *B. intermedia* and three of its lepidopteran pupal hosts: gypsy moth, greater wax moth, *Galleria mellonella* (L.) (Pyralidae), and spruce budworm, *Choristoneura fumiferana* (Clemens) (Tortricidae), have shown that *B. intermedia* responds best to kairomones extracted from pupae of the gypsy moth (Tucker and Leonard 1977). Spruce budworm pupae were of intermediate attractiveness. The same preference for gypsy moth pupae was observed by Minot and Leonard (1976b).

Host diet may play a role in the fitness of a parasitoid (Leius 1961). Thus, species of hosts that can be reared in the laboratory on artificial diet should be compared to hosts reared on other diets including plant material. Greenblat and Barbosa (1981) found that the largest *B. intermedia* emerged from pupae of gypsy moths that had fed upon foliage of red oak, *Quercus rubra* L. (Fagales: Fagacaea). The overall size of the parasitoids, however, did not correlate with the size of the host. Hosts reared on a synthetic diet were larger than hosts reared on red oak, but *B. intermedia* reared on the pupae of hosts fed synthetic diet were smaller than those parasitoids reared from hosts fed red oak. Hosts reared on synthetic diet were as suitable for rearing *B. intermedia* as the pupae of moths reared from grey birch, *Betula populifola* Marsh (Fagales: Betulaceae), white oak, *Quercus alba* L. (Fagacaea), or red maple, *Acer rubrum* L. (Sapindales: Aceraceae). The pupae of wax moths were found to be of

intermediate suitability (Greenblat and Barbosa 1981), yet *B. intermedia* developed in the least amount of time on this species (Minot and Leonard 1976b).

Diets of adult parasitoids also should be evaluated in insectary rearing programs. Leius (1961) showed that fecundity and longevity of adults of the parasitoid *Itoplectis conquisitor* (Say) (Hymenoptera: Ichneumonidae) were greatly influenced by diet. The greatest number of eggs were produced by females feeding on pollen, honey, and the body fluids of the host. Carbohydrates were found to be necessary for the sustained production of eggs.

Some species of parasitoids are host specific while other species may be reared on alternate hosts (Marston and Ertle 1973). Problems associated with rearing parasitoids on host species other than their natural host can include a decrease in development to adulthood and decreased fitness as an adult. For example, adult *B. intermedia* reared on pupae of the greater wax moth were smaller than those reared on their primary host, the gypsy moth (Rotheray et al. 1984). Associated with the reduction in size was a reduced ability of the adult to parasitize the larger gypsy moth pupae. Marston and Ertle (1973) found several differences in the rearing of *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae) on eggs of either cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), or the angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae). One difference was that parasitoids reared from *T. ni* eggs searched for hosts in a greater area than parasitoids reared from

S. cerealella eggs. However, the use of *S. cerealella* eggs would be beneficial in a biological control program where the cost of production is important. *S. cerealella* are less expensive based on the cost of host production and correspondingly the cost per meter traveled by the parasitoid when searching (Marston and Ertle 1973).

Insectary-reared insects usually must adapt to a laboratory environment by varying degrees. This adaptation, along with extensive inbreeding, can lead to reduced fitness in the field and a failure of the use of parasitoids in biological control programs (Metcalf and Metcalf 1982). Ng and coworkers (1985) compared laboratory and field strains of *B. intermedia* and found that the field strain parasitized more hosts in a shorter period of time than the laboratory strain. However, over a longer exposure period, they parasitized a similar number of hosts. In the field, this longer period for oviposition may be detrimental as one generally desires to have the greatest number of pests parasitized in the shortest time period especially if one is trying to control the egg or pupal stage of a pest. Although insectary-reared insects may lose some characteristics that are desirable in the field, certain characteristics may be selected in the laboratory that would increase the parasitoid's effectiveness in a particular biological control program. For example, an increased percentage of females may be desirable in an inundative release program (Roush 1979).

Inbreeding is often believed to be undesirable, but may not always present a problem. Certain parasitoid species may be adapted to inbreeding, as

the potential exists for sib-mating in the field among many gregarious parasitoids (Waage and Greathead 1986). Hoy (1977) found no detectable difference in female longevity or fecundity after nine generations of inbreeding by the arrhenotokous predatory mite *Metaseiulus occidentalis* (Nesbitt) (Acari: Phytoseiidae), and the progeny sex ratio and viability were not affected. This same trend also may exist for parasitoids. Waage and Greathead (1986) noted that parasitoid species with female-biased sex ratios may be adapted to inbreeding while those with an even sex ratio may not be adapted to inbreeding.

Sex ratio is an aspect of insectary rearing that must be carefully monitored. Sex ratio is regulated by a variety of factors, not all of which may be identified. King (1987) listed 14 factors that affect parasitoid offspring sex ratios. The various factors may play a role in the conclusions of certain studies. For example, Barbosa and Frongillo (1979a) noted that parasitism of small wax moth hosts by *B. intermedia* resulted in a greater proportion of male progeny. This result was later contradicted by Greenblat and Barbosa (1981), who utilized gypsy moth pupae as hosts. The sex ratio for *B. intermedia* is generally female skewed (Mohamed and Coppel 1986). A female skewed sex ratio also has been demonstrated for *B. ovata*. Great variation in sex ratio for *B. ovata*, while maintaining a female skew, was noted by Grant and Shepard (1987) who utilized freezer-stored *A. gemmatalis* pupae as hosts. Sex ratio regulation by female *B. intermedia* was found to be a function of male population density (Mohamed and Coppel 1986). When the parental sex ratio was male skewed, the progeny were

female skewed to a greater extent then when the parental sex ratio was female skewed. They also found that no correlation existed between host size and the sex of the resulting progeny. Another factor in the sex ratio of offspring is the age of the maternal parent at the time of oviposition, or time since insemination (Barbosa and Frongillo 1979a). One factor that can lead to a male skewed sex ratio under colony conditions for *B. ovata* is a majority of unmated females caused by overcrowding in the colony cage. When overcrowding occurs, courting pairs are often interrupted by other individuals before copulation (personal observation). As *B. ovata* is an arrhenotokous parasitoid, unmated females will produce only male offspring.

Brachymeria ovata fits the definition of parasitoid as discussed earlier in this chapter with some additional characteristics. The female can be responsible for the death of more pupal hosts than those in which she deposits her eggs. Females of *B. ovata*, like many other parasitoids, may utilize the pupa as a food source, as well as a host for its offspring. When the pupa is utilized as a food source, the female will pierce the pupa with her ovipositor, extract it, and then feed on exuding fluids. This feeding process may kill the host pupa, without the female actually laying an egg. In some cases the pupa may be an unacceptable host to the female, and she neither oviposits, after inserting her ovipositor, nor feeds. This phenomenon has been termed parasitoid-related mortality (Minot and Leonard 1976b). Parasitoid-related mortality may not occur in all hosts of *B. ovata*. L. O. Howard observed a female *B. ovata* pierce a pupa of a white-

marked tussock moth, *Orgyia leucostigma* (J. E. Smith) (Lepidoptera: Lymantriidae), three times, and on the third time hold her ovipositor within the pupa for more than two minutes, presumably ovipositing within it. One week later, an adult moth emerged from the pupa (Howard 1897). The tussock moth may have the ability to encapsulate the parasitoid egg, or may not be affected by any arresting factor that may be associated with parasitoid oviposition. Encapsulation is an immune response by an insect involving primarily plasmocytes surrounding the parasitoid or its egg, melanizing and killing it (Woodring 1985). Encapsulation of parasitoid eggs can reportedly occur in some host species while it does not occur in other host species parasitized by the same species of parasitoid (Askew 1971).

Brachymeria species parasitize a wide range of lepidoptera. The genus *Brachymeria* contains 27 species in America north of Mexico (Burks 1960). Five species of *Brachymeria* are primary parasitoids of lepidoptera, and two species are secondary parasitoids of tachinid flies that attack lepidoptera. Two other species parasitize the tachinid and sarcophagid parasitoids of grasshoppers, and one species parasitizes blow flies. Others are either primary or secondary parasitoids.

Brachymeria ovata is a primary endoparasitoid of a wide range of lepidopteran pupae. The distribution of *B. ovata* extends from Northern Mexico, throughout the United States except Montana, North Dakota, and Northern New England, and is recorded only from Ontario in Canada (Burks 1960). Lengths of

males and females range widely (from 3.5 to 5.0 mm and from 3.5 to 6.5 mm for males and females, respectively). Females can be distinguished from males by the presence of a black band completely encircling the hind femur of the female, whereas in the male the band is broken on the posterior portion of the femur. The pubescence of females is silvery, while the pubescence of males is typically golden on the thoracic dorsum and silvery elsewhere.

Hosts of *B. ovata* include 18 families of lepidoptera from the microlepidoptera to large butterflies such as monarchs and swallowtails (Burks 1960). Exposed pupae or chrysali may be parasitized more often than pupae within cocoons. In the field, Johnson (1983) reported a higher percentage parasitism of exposed pupae of the american cotton leafworm, *Alabama argillacea* (Lepidoptera: Noctuidae), compared to pupae encased within cotton leaves (30.3 to 46.6% and 1.0 to 4.9%, respectively). In the laboratory, pupae of *T. ni* also are parasitized to a greater extent when removed from their silken cocoons (unpublished data). *B. ovata* reportedly does not crawl into crevices but locates pupae while flying, and chooses pupae that are larger than 1.5 cm (Krombein et al. 1979). However, females will oviposit in pupae smaller than 1.5 cm (personal observation). The behavioral sequence of oviposition of *B. intermedia* was described by Tucker and Leonard (1977). *B. ovata* follows a similar if not identical pattern (Grant and Noblet 1991).

Brachymeria ovata adults have been observed in the field during the months of July through October (Burleigh 1971, Howard 1897). Howard also

reported that no *B. ovata* were reared from cocoons collected in the spring or the fall, and concluded that *B. ovata* overwinters as an adult. *B. intermedia*, a closely related species, has been found overwintering in forest litter (Ticehurst et al. 1978), under the loose bark of dead trees (Dowden 1935), and within wood borer tunnels in a dead chestnut oak, *Quercus prinus* L. (Fagales: Fagaceae) (Waldvogel and Brown 1978). Parasitoids within borer tunnels were found in groups of 5 to 15, and a few were found singly. Many of the adults had been apparently preyed upon by spiders. Those adults in the center of groups may survive better than those along the edges as they are protected from predation and are better insulated from environmental conditions. Adult size also may play a role in success of overwintering (Greenblat and Barbosa 1981) as larger individuals are able to store greater energy supplies.

Aggregation of *Brachymeria lasus* (Walker) (Hymenoptera: Chalcididae) has been studied by Simser and Coppel (1980). Within colony cages, these parasitoids congregated overnight within hollow styrofoam cubes that had previously been exposed to adult conspecifics. These data provided evidence for a pheromonal basis for aggregation. A pheromone, identified as 3-hexanone, responsible for aggregation in *B. intermedia* was isolated from both sexes (Mohamed and Coppel 1987b). Pheromones also may be important in overwintering as well as in the selection of overnight resting places (Simser and Coppel 1980).

In the laboratory, *B. ovata* has been reared on about 10 species of moths. Patana et al. (1978) reported on its development in six hosts: pink bollworm, *Pectinophora gossypiella* (Saunders) (Gelechiidae); *T. ni*; beet armyworm, *Spodoptera exigua* (Hübner) (Noctuidae); tobacco budworm, *Heliothis virescens* (F.) (Noctuidae); cotton bollworm, *Helicoverpa* (=*Heliothis*) *zea* (Boddie) (Noctuidae); and the saltmarsh caterpillar, *Estigmena acrea* (Drury) (Arctiidae). Grant and Shepard (1987) reared *B. ovata* from freezer-stored pupae of the noctuids: soybean looper, *Pseudoplusia includens* (Walker); fall armyworm, *Spodoptera frugiperda* (J. E. Smith); and *A. gemmatalis*; as well as *T. ni, S. exigua, H. virescens*, and *H. zea*. *B. ovata* also has been reared on a limited number of *G. mellonella* (unpublished data).

Under colony conditions, *B. ovata* will superparasitize their hosts, that is, lay more than one egg per pupa. One female may lay more than one egg per pupa (self-superparasitism), or several females may lay one or more eggs per pupa. At times, two or three females may simultaneously oviposit in a single host. Researchers have noted that first-instar larvae of solitary endoparasitoids, such as *B. ovata*, will bite one another when more than one egg hatches per host (Askew 1971). Under these conditions, usually only one parasitoid survives. Dissections of superparasitized *T. ni* hosts of *B. ovata* showed that when more than one egg hatches per host, only one larva remains alive after several days (personal observation). Dead *B. ovata* larvae will have dark marks on them, presumably caused by the mandibles of another larva.

Self-superparasitism among solitary parasitoids, such as *B. ovata*, can be advantageous (van Alphen and Visser 1990). More than one offspring per host could increase the chance that the superparasitizing parent may produce an adult offspring if a conspecific or other parasitoid later parasitizes the same host. Another situation in which self-superparasitism may be advantageous is when the host can encapsulate the parasitoid egg. With two or more eggs per host, the host may be unable to encapsulate all of the eggs. In such cases of self-superparasitism it would be advantageous if the parasitoid laid more than one egg per insertion of her ovipositor (van Alphen and Visser 1990). As pupae parasitized by *B. ovata* often contain only one egg, it is unlikely that *B. ovata* lays more than one egg per insertion of the ovipositor (personal observation). Under laboratory rearing conditions, superparasitism may not be advantageous when rearing *B. ovata*, especially when utilizing freezer-stored pupae.

As stated earlier, the use of freezer-stored pupae may be a suitable means of acquiring large numbers of parasitoids for release in biological control programs. However, information on the effects of the use of freezer-stored pupae for rearing *B. ovata* with regards to biological characteristics is limited, and the effects of using freezer-stored pupae on reproductive characteristics of *B. ovata* have not been investigated. Detrimental effects caused by freezer storage would limit its successful utilization as a method of rearing large numbers of parasitoids for use in biological control programs or laboratory studies.

The overall objective of this research was to determine the influence of using freezer-stored pupae as hosts on biological, mainly reproductive, characteristics of *B. ovata*. The specific objectives of research reported herein were to: I) examine the mating and ovipositional biology of *B. ovata*, specifically: 1) assess mating abilities of *B. ovata* reared from freezer-stored pupae (i.e., will they readily mate and compete with 'normal' *B. ovata* reared from live pupae?), 2) determine the preovipositional period of *B. ovata* reared from live and freezer-stored pupae, and 3) determine the effect of time of day on oviposition of *B. ovata* reared from live pupae; II) determine the influence of the use of freezer-stored pupae as hosts on reproductive capabilities of *B. ovata*, examined were: 1) progeny production, 2) longevity and fecundity, and 3) correlation between body size and longevity, fecundity, or progeny production; and III) examine selected biological characteristics, including: 1) pupal acceptability (i.e., eggs laid inside the host) and suitability (i.e., progeny produced) of live and freezer-stored hosts, 2) the length of development of immature stages in live and freezer-stored pupae, and 3) the optimal pupal age for use as freezer-stored hosts.

Chapter II

MATING AND OVIPOSITIONAL BIOLOGY OF *Brachymeria ovata*

Introduction

The success of a biological control program utilizing periodic releases of parasitoids may depend on the ability of the insectary-reared parasitoids to mate with resident "wild" individuals. Success in mating requires a proper sequence of behaviors in the courtship pattern as well as the presence of chemical cues. A pheromonal basis to courtship behavior for two chalcidid species, *Brachymeria intermedia* (Nees) and *B. lasus* (Walker), has been identified (Mohamed and Coppel 1987a), and the courtship pattern for *B. intermedia* is well documented (Leonard and Ringo 1978).

An understanding of the preovipositional period and peak adult activity is essential for correct timing of parasitoid releases in a biological control program. This information also can be used to optimize insectary rearing, as well as to provide a better understanding of the insect's biology. Studies of *B. intermedia* have determined that adult parasitoids are most active in the afternoon between 1330 and 1530 h (Minot and Leonard 1976a). *Brachymeria ovata* (Say) also is generally more active in the afternoon (J. F. Grant, personal communication). Utilizing kairomones, Tucker and Leonard (1977) found that responses by *B. intermedia*

peaked between 1000 and 1200 h, and activity was high between 0900 and 1400 h.

The use of freezer-stored pupae for rearing parasitoids provides an additional method for increased utilization of parasitoids in biological control programs. The use of freezer-stored pupae should not detrimentally impact upon the reproductive abilities of the parasitoids if this method is to be used successfully. Little information is available on the impact of freezer-stored pupae, used as hosts, upon parasitoids.

The objectives of research reported herein were to: 1) assess the mating abilities of *B. ovata* reared from freezer-stored pupae as compared to *B. ovata* reared from live pupae and crosses of the two, 2) determine the preovipositional period for *B. ovata* females reared from freezer-stored pupae and live pupae, and 3) determine the time of maximum oviposition for *B. ovata* reared from live pupae.

Materials and Methods

Colony Maintenance. A colony of *B. ovata* was established from adult females collected at the University of Tennessee, Plant Science Field Laboratory, Knoxville, TN, in August and September, 1989. The cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), was used for both maintenance of the colony and experimental procedures. *T. ni* was selected as a host because of past reports of successful rearing of *B. ovata* from live and freezer-stored pupae of *T. ni* (Patana et. al. 1978, Grant and Shepard 1987) and because of the ease of rearing this host in the laboratory. Adult *B. ovata* were held in Plexiglas® cages (31 x 31 x 41.5 cm)

with screened openings (12 cm) in three sides and a sleeve fitted to an opening (12 cm) in the forth side. Several crumpled paper towels were placed on the floor of the cage to provide resting areas. The colony was provided with a constant supply of water and a mixture of sucrose saturated with honey. Approximately 100 *T. ni* pupae (2 to 4 days old) were exposed three times each week to maintain the colony. Exposed pupae were held in petri dishes and emergent adult parasitoids were added daily to colony cages. All colonies were held, and all experiments conducted, at 27±2°C, 50-65%RH, in a laboratory or an incubator with a photoperiod of 14:10 (L:D) except where noted.

The colony of *T. ni* was reared on a pinto bean lepidopteran diet from Shorey and Hale (1965). One gram of aureomycin was added to each liter of this diet. Approximately 30 ml of diet were poured into waxed-paper cups (88.7 ml) and stored at 10°C for as long as one week before use. Fifteen to 20 newly emerged larvae were placed into cups using a number "3" camel's hair brush. Cups were covered with a square of paper towel which was secured with a rubber band. Fourteen days later, and every other day thereafter, the pupae were removed from the cups until all had been removed. Pupae were maintained at 25°C for one day to ensure that all pupae were more than 24 hours old prior to use in experiments or use in maintaining the parasitoid colony. Approximately three times each week, 50 pupae were placed in 3.8 l glass jars with a piece of paper towel covering the floor and the mouth of the jar, and a 5 cm strip of paper towel was suspended inside the jar from the mouth to provide additional area for oviposition. Lids and

strips were replaced every other day and held in plastic boxes (17 x 12 x 6.5 cm) until eclosion of eggs. A 20% sucrose solution was provided to adult moths and replaced every other day.

Unless otherwise stated, freezer-stored pupae of *T. ni* were obtained by placing live pupae (2 to 4 days old) in petri dishes (100 x 15 mm) in a manual-defrost freezer (maintained at -20°C) for 30 to 90 days. Before exposure to female *B. ovata*, freezer-stored pupae were spread on paper towels and thawed at room temperature for 4 to 6 hours.

Mating Abilities of Adults Reared from Freezer-stored Pupae. Mating abilities were assessed by pairing adult *B. ovata* reared from live and freezer-stored pupae of *T. ni* in varying combinations in petri dishes (100 x 15 mm). A filter paper (7 cm) was placed on the bottom of each petri dish. Combinations included: 1) one male and one female reared from a freezer-stored pupa, 2) one male and one female reared from a live pupa, 3) one male reared from a live pupae combined with one female reared from a freezer-stored pupa, and 4) one male reared from a freezer-stored pupa combined with one female reared from a live pupa. Males were newly-emerged to one day old and females were newly-emerged. Pairs in petri dishes were exposed to ten live pupae per day for one week. Pupae were exchanged daily and exposed pupae were maintained at 27°C in petri dishes until parasitoid emergence. The sex of emerging parasitoids was examined daily, and successful matings were determined by the production of female offspring. As *B. ovata* is an arrhenotokous parasitoid, success of matings could be determined by

the sex of the offspring. Three blocks of 10 pairs were examined for each combination.

Preovipositional Period. The preovipositional period was determined by placing one newly-emerged female with one newly-emerged or one-day-old adult male in a petri dish (100 x 15 mm). One *T. ni* pupa was provided and monitored daily until oviposition marks on the pupa were observed. The pupa was replaced if its eyes turned dark, indicating the adult moth was nearly developed. Combinations examined included: 1) one male and one female reared from freezer-stored pupae, 2) one male and one female reared from live pupae, 3) one male reared from live pupae combined with one female reared from freezer-stored pupae, and 4) one male reared from a freezer-stored pupa combined with one female reared from a live pupa. Three blocks of 10 pairs were examined for each combination.

Effect of Time of Day on Oviposition. To determine the daily ovipositional pattern of *B. ovata*, 15 females, 10 to 20 days old, were placed in separate petri dishes (100 x 15 mm), each with ten pupae of *T. ni*. Pupae were replaced every four hours for 24 hours and held for adult parasitoid or moth emergence. During periods of darkness, a small flashlight with a red filter was used to provide enough light to replace pupae. Exposure of *B. ovata* to pupae began at midnight. A photoperiod of 16:8 (L:D) was chosen to obtain two, four-hour periods of dark, and four, four-hour periods of light. The number of *B. ovata* progeny as well as the number of dead pupae (i.e., pupae not producing adult moths) for each time period

for each adult was recorded. One hundred pupae (i.e., control) were held separately and were not exposed to parasitoids. This entire experiment was repeated three times.

Female Reproductive System. Five females from the parasitoid colony were dissected to determine the structure of the reproductive organs. Dissections were performed on freeze-killed individuals that had been previously observed to oviposit in hosts.

Data Analysis. Data were analyzed by Student's t test or by analysis of variance and Duncan's (1955) multiple range test where appropriate (SAS Institute 1985). Arcsin transformations of percentages were conducted prior to statistical analysis. A 0.05 level of probability was used to determine significant differences.

Results and Discussion

Mating Abilities of Adults Reared from Freezer-stored Pupae. Freezer-storage of *T. ni* hosts did not greatly impair the mating abilities of *B. ovata* adults. Both males and females reared from freezer-stored pupae readily mated with adults reared from live or freezer-stored pupae (Table 1). Most (70%) of the females reared from live or freezer-stored pupae and paired with males reared from live pupae produced both male and female offspring (Table 1). A reduction in percentages (43-53) of females producing female offspring was observed when their male companions had been reared from freezer-stored pupae. While not statistically significant, this reduction could upset sex ratios of progeny in a rearing

Table 1. Mating abilities of combinations of *Brachymeria ovata* reared on live and freezer-stored *Trichoplusia ni* pupae.

Mated Pairs			Offspring Produced		% Producing Female Offspring
Female		Male	Female	Male	
Live	X	Live	Yes	Yes	70a[a,b]
Freezer-stored	X	Freezer-stored	Yes	Yes	43a
Live	X	Freezer-stored	Yes	Yes	53a
Freezer-stored	X	Live	Yes	Yes	70a

[a] Values within a column followed by the same letter are not significantly different ($P > 0.05$, Duncan's multiple range test); arcsin transformation was perfomed on the percentages before data analysis.
[b] n=45.

program, as unmated females produce only male offspring. This reduction in the percentage producing female offspring may be due to mold growth, as mold was present in some of the parasitized freezer-stored pupae. Further studies are needed to determine if the effects of freezer-stored pupae or mold contamination are responsible for the decrease in mating success, relative to the percentage of females producing female offspring, among individuals reared from freezer-stored pupae. As 29 out of 30 pairs of *B. ovata* reared from freezer-stored pupae in the progeny production study (see Chapter III) produced both male and female progeny, the rearing of *B. ovata* on freezer-stored pupae alone is probably not responsible for the reduction in percentages of pairs successfully mating in this study.

Preovipositional Period. The preovipositional period for all *B. ovata* tested ranged from two to nine days (Table 2). This range represents a much smaller one than reported by Patana (1979), who reported a range of 5 to 105 days at 25°C, and 3 to 21 days at 30°C, with an average of 33.5 and 6.9 days, respectively. However, a variety of host species were used in his studies. Differences in the range of preovipositional periods between this study and that of Patana (1979) also may be due to biological differences in the populations from which the parasitoids were collected to establish the colony.

When one male or female of a pair was reared from freezer-stored pupae, the ovipositional period of the female increased. The mean preovipositional period of females reared from freezer-stored pupae paired with males reared from live pupae was significantly greater than that of pairs in which both individuals were

Table 2. Influence of freezer storage on preovipositional period of females, among *Brachymeria ovata* pairs.

B. ovata Pairs Reared From:			Preovipositional Period	
Female		Male	$\bar{x}$ Days ± S.D.	Range
Live	x	Live	4.37 ± 0.89a[a,b]	2-6
Freezer-stored	x	Freezer-stored	4.57 ± 1.28ab	2-8
Live	x	Freezer-stored	4.93 ± 1.20ab	3-8
Freezer-stored	x	Live	5.10 ± 1.45b	3-9

[a] Values within a column followed by the same letter are not significantly different ($P > 0.05$, Duncan's multiple range test).
[b] n = 45.

reared from live pupae (Table 2). The lengthened mean preovipositional periods for females reared from freezer-stored pupae, or for those females paired with males reared from freezer-stored pupae, may be due to a slowing in the maturation of adults reared from freezer-stored pupae, just as development in the preadult stages is slowed when utilizing freezer-stored pupae as hosts (see Chapter IV).

Effect of Time of Day on Oviposition. The mean number of pupae parasitized per four hours during a 24-hour period is illustrated in Figure 1. The number of dead pupae represents all those that did not produce adult moths; this number includes pupae in which an egg was laid and those killed by parasitoid-related mortality. The number of emergent *B. ovata* represents the number of individuals that emerged from the dead pupae. The highest level of parasitism (68.7%) occurred during the first four-hour period of light (0800 to 1200 h). During the next three periods of light, the number of pupae in which the females inserted their ovipositors, as indicated by the number of dead pupae, leveled off at an average of between five and six pupae. The number of adult *B. ovata* emerging from those pupae decreased steadily throughout the day. This decrease throughout the day may be a result of the rate of production of eggs within the ovaries. Low numbers of pupae were parasitized during the periods of darkness, probably as a result of the parasitoids being disturbed as pupae were replaced in the petri dishes. Otherwise, *B. ovata* remained essentially motionless in the dark as observed under short periods of low intensity red light. All 100 pupae held separately and not

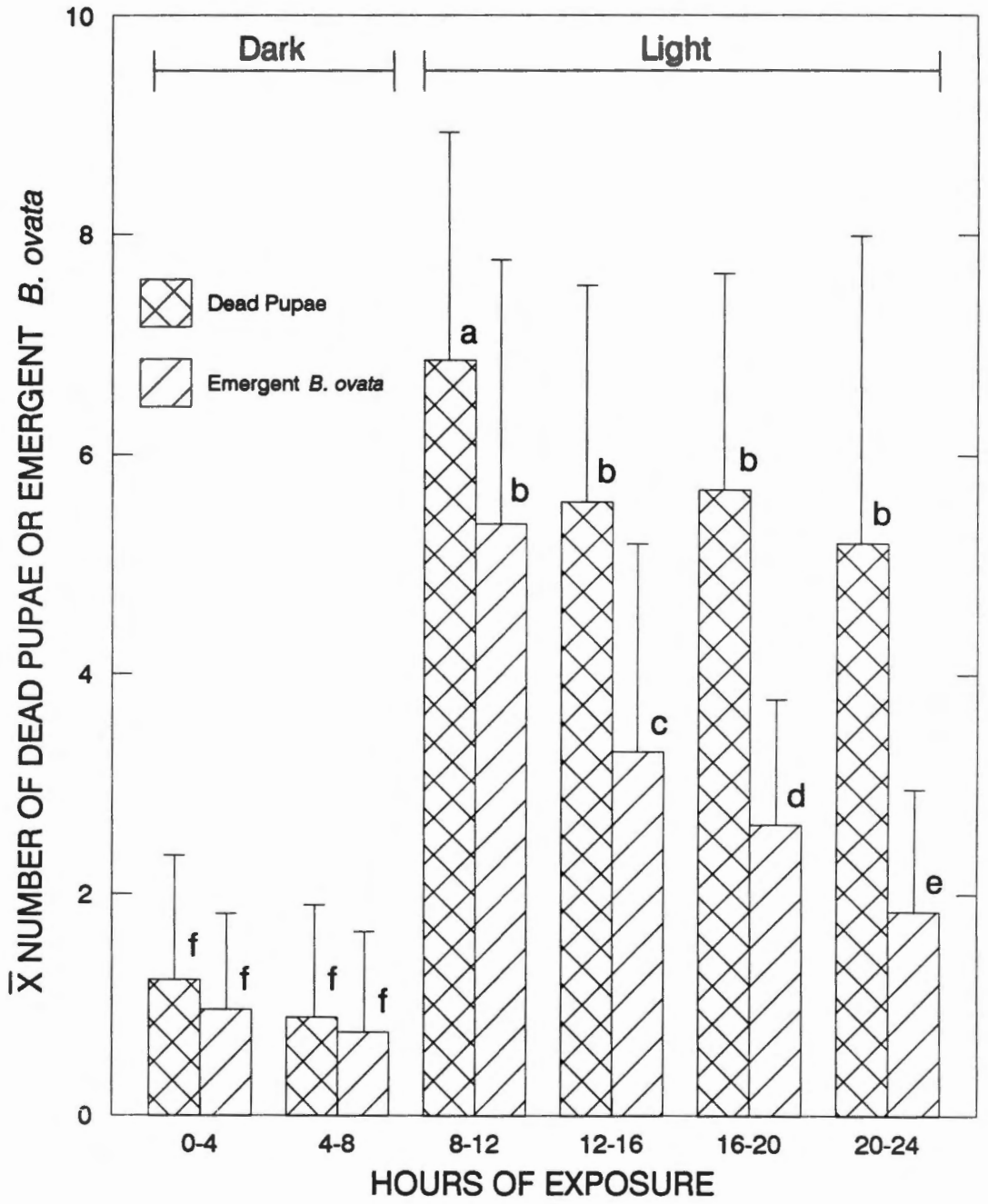

Figure 1. Daily ovipositional pattern of *Brachymeria ovata* exposed to live *Trichoplusia ni* pupae. Dead pupae include those parasitized and those killed by parasitoid-related mortality. Bars with the same letter are not significantly different ($P > 0.05$, Duncan's multiple range test, lines represent S.D.).

exposed to parasitoids developed into adult moths, therefore no adjustments to the number of dead pupae were necessary.

Female Reproductive System. The maximum number of progeny that was produced during one 24-hour period was 30, supporting data on rates of parasitism by Patana (1979). Dissections of female *B. ovata* determined that each female has two ovaries each with three ovarioles. Therefore, each ovariole must produce at least five eggs per day during peak oviposition. The number of eggs produced per day is a major biological difference between *B. ovata* and *B. intermedia*. *B. intermedia*, while possessing the same number of ovarioles, produces only six eggs per day (Barbosa and Frongillo 1979a).

The use of freezer-stored pupae of *T. ni* as hosts of *B. ovata* did not detrimentally impact upon the biological characteristics examined. It is believed by the author that the reduction in successful mating of males reared from freezer-stored pupae is not entirely due to rearing the parasitoids from freezer-stored pupae. While freezer-stored pupae may have impacted upon the preovipositional period, the observed increase is not large when compared to preovipositional periods observed in other studies (Patana 1979).

Chapter III

COMPARATIVE REPRODUCTIVE CAPABILITIES OF *Brachymeria ovata*

Introduction

Life history traits of a parasitoid species are important parameters to investigate when choosing a laboratory host (Chambers 1977). Host-related differences in adult parasitoids reared on different host species can produce apterous adults in a normally winged species (Salt 1941) or adults that search a smaller area for hosts when reared on one host species as opposed to another (Marston and Ertle 1973).

Longevity and fecundity are important parameters to consider, in regard to the number of hosts a female can parasitize and her ability to overwinter, when releasing parasitoids in the field. Fecundity can be affected by environmental factors such as photoperiod and temperature, as well as host quality (Askew 1971). Resorption of eggs within the ovaries of parasitoids can occur when insects avoid adverse conditions such as during overwintering or aestivation (Bell and Bohm 1975). Resorption of eggs within the ovaries of the chalcidid *Brachymeria intermedia* (Nees) occurred at temperatures between 15 and 21°C, and short photoperiods (10-12 h) enhanced this resorption (Barbosa and Frongillo 1979b). High temperatures also may have an adverse affect on

the reproductive capabilities of parasitoids. For example, *Brachymeria ovata* (Say) males reared at, or adult males exposed to, 35°C were found to be sterile (Patana 1979).

Progeny production is important for the continued survival of successive generations in the field and for an efficient laboratory rearing program. Progeny production of *B. ovata* was found to be influenced by temperature, as females produced between 47 and 309 offspring at 25°C and between 94 and 362 offspring at 30°C (Patana 1979). However, only 5 to 101 progeny were produced at 35°C.

Fecundity and progeny production can be affected by the size of an adult parasitoid (Askew 1971, Rotheray et al. 1984). Smaller *B. intermedia* reared on pupae of the greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae), were not as able to overcome the host defenses, and therefore parasitized fewer hosts in the same time period as individuals reared on pupae of the gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae) (Rotheray et al. 1984).

The objectives of research reported in this chapter were to: 1) determine the longevity of unmated individuals not exposed to pupae and mated pairs exposed to live or freezer-stored pupae, and the fecundity of *B. ovata* reared from live or freezer-stored pupae and exposed to live or freezer-stored pupae, 2) determine the progeny production of mated female *B. ovata* reared from live or freezer-stored pupae and exposed to live or freezer-stored pupae, and

3) determine any correlation that may exist between the body size of *B. ovata* and longevity, fecundity, or progeny production.

Materials and Methods

The host colony and *B. ovata* colony were maintained as outlined in Chapter II. Experiments were conducted in a laboratory or an incubator maintained at 27±2°C, 50 to 65%RH and a photoperiod of 14:10 (L:D). The cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), was used as hosts in all experiments. Unless otherwise stated, freezer-stored pupae of *T. ni* were obtained by placing live pupae (2 to 4 days old) in petri dishes (100 x 15 mm) in a manual-defrost freezer (maintained at -20°C) for 30 to 90 days. Before exposure to female *B. ovata*, freezer-stored pupae were spread on paper towels and thawed at room temperature for 4 to 6 hours.

Longevity and Fecundity. Longevity and fecundity of adult *B. ovata* reared from live or freezer-stored pupae were determined for: 1) individual males and females reared from live pupae, 2) individual males and females reared from freezer-stored pupae, 3) males reared from live pupae paired with females reared from live pupae, and 4) males reared from freezer-stored pupae paired with females reared from freezer-stored pupae. Newly-emerged adult *B. ovata* were held separately or paired in petri dishes (100 x 15 mm), with one piece of filter paper (9 cm) placed in it. Honey was streaked on the undersurface of the lid as a food source. Pairs were provided with either four

live or four freezer-stored pupae daily until death of the female parasitoid. A similar method was used to determine the fecundity of *Itoplectis conquisitor* (Say) by Arthur (1963). Pupae were placed in plastic cups (30 ml) and stored in the freezer (at -20°C) after exposure to adults. The contents of each pupa were later examined individually using a Wild stereoscope to determine the fecundity of each female. Pupae were divided lengthwise and the contents of each pupa were spread on a petri dish using forceps. Eggs of *B. ovata* could be easily observed within the pupal contents. The number of eggs in each of the four pupae exposed per day were recorded. Three replications of five individuals or pairs in each treatment were conducted (total of n=15/treatment). Only one replication each for females reared from live pupae and females reared from freezer-stored pupae (total n=5/treatment) exposed to freezer-stored pupae and evaluated for fecundity will be presented and discussed due to the long time necessary to dissect the many freezer-stored pupae.

Progeny Production. To determine the influence of freezer storage on progeny production, male and female *B. ovata* reared from freezer-stored pupae were paired and then exposed daily to live or freezer-stored pupae. As a comparison, male and female *B. ovata* reared from live pupae also were paired and then exposed daily to live or freezer-stored pupae. One newly-emerged female and one male (newly-emerged to 1-day-old) were paired in a petri dish (100 x 15 mm), with honey streaked on the undersurface of the lid as a food source. Four days later, 30 live or freezer-stored pupae were provided as hosts

for 24 hours. Live pupae were replaced daily until death of the female (average lifetime of female *B. ovata* exposed to live pupae is about 15 days). Freezer-stored pupae were provided and replaced daily for 14 days. This period was chosen because longevity studies have shown that females exposed to freezer-stored pupae can live as long as 132 days, and limited numbers of freezer-stored pupae were available. Exposed pupae were held at 27°C and examined daily for adult parasitoid or moth emergence (from live pupae). The number of male and female *B. ovata* progeny and number of moths were recorded. Means were determined by dividing the total progeny produced on each day of oviposition by the number of females alive on that day. Three replications of five pairs in each treatment were conducted (total n = 15 pairs/treatment).

Adult Size. Individual *B. ovata* obtained from the previous two studies were preserved individually in vials (5 ml) containing 70% ethanol. One forewing and one mesothoracic tibia from each individual were mounted on microscope slides and measured with an ocular micrometer in an American Optical stereo microscope. Correlation analysis between wing and tibia measurements and longevity and progeny production was conducted using SAS procedure "corr" (SAS Institute 1985).

Data Analysis. Data were analyzed by Student's t test or by analysis of variance and Duncan's (1955) multiple range test where appropriate (SAS Institute 1985). Arcsin transformations of percentages were conducted prior to

evaluation. A 0.05 level of probability was used to determine significant differences.

Results and Discussion

Longevity and Fecundity. The longevity of individual unmated female *B. ovata* adults reared from freezer-stored pupae differed significantly from those adults reared from live pupae and males reared from freezer-stored pupae (Table 3). Individual females generally lived longer than individual males. Individual females may be able to survive longer as they are generally larger and therefore may have greater energy reserves in the form of fats and proteins. Individuals reared from freezer-stored pupae may live longer as a result of the lack of a detrimental substance that may be present in the live pupae. This substance may be a chemical obtained from the larval diet and carried over to the pupal stage. It also may be enzymes, hormones or molting fluid present in the live pupa that may be destroyed by freezing and subsequent thawing.

Longevity of females exposed to, and ovipositing in, freezer-stored pupae was significantly longer (approximately four times) than females exposed to live pupae (Table 4). This increase in longevity may be due to the lack of a compound detrimental to the longevity of females exposed to live pupae. Females often feed on pupal fluids exuding from the oviposition hole in the pupa. Freezer storage and subsequent thawing of pupae may break down a toxic compound; conversely, there may be a breakdown product in freezer-stored

Table 3. Longevity of individual unmated adult *Brachymeria ovata* adults not exposed to *Trichoplusia ni* pupae.

Adults Reared from:	Sex[a]	Longevity ($\bar{x} \pm$ S.D.)
Live Pupae	Male	101.0±25.6a[b] 60-130 (14)[c]
	Female	114.0±27.2a 60-150 (15)
Freezer-stored Pupae	Male	100.4±20.6a 63-135 (14)
	Female	135.8±24.9b 90-174 (13)

[a] Sex of adults reared from live or freezer-stored pupae.
[b] Values followed by the same letter are not significantly different ($P > 0.05$, Duncan's multiple range test).
[c] Range of longevities (n).

Table 4. Longevity of adult *Brachymeria ovata* reared from live or freezer-stored pupae of *Trichoplusia ni* and exposed to live or freezer-stored pupae.

B. ovata Reared from:	*B. ovata* Exposed to:	Sex	Longevity ($\bar{x} \pm$ S.D.)
Live Pupae	Live Pupae	Male	75.8±34.7a[a]
		Female	14.6±2.1b
	Freezer-stored Pupae	Male	74.7±35.2a
		Female	60.4±21.4a
Freezer-stored Pupae	Live Pupae	Male	86.5±34.7a
		Female	15.6±4.1b
	Freezer-stored Pupae	Male	76.5±30.2a
		Female	68.5±26.7a

[a] Values within a column followed by the same letter are not significantly different ($P > 0.05$, Duncan's multiple range test).

pupae that is beneficial to female *B. ovata*. Male longevity was not affected by the type of pupae to which they were exposed (Table 4). Males have not been observed to feed on pupal fluids (personal observation), thus supporting the possibility of a detrimental substance present in live pupae.

All female *B. ovata* reared from live and freezer-stored pupae and exposed to live pupae died within 23 days and produced no more than 197 eggs (n=27) (Tables 4 and 5). Females exposed to freezer-stored pupae lived as long as 129 days and produced as many as 706 eggs (Tables 4 and 5). Of the five individual *B. ovata* reared on and exposed to freezer-stored pupae, only one adult died within 19 days and produced 157 eggs. The remaining four females lived longer than 70 days and produced more than 400 eggs each. These females produced more than 200 eggs within the first 16 days of oviposition, and more than 400 eggs by day 30 of oviposition. By day 50, all females had produced greater than 97% of their total fecundity. Daily averages of eggs produced by these females exposed to freezer-stored pupae were not lower than four eggs per day until day 40 of oviposition. Daily averages of females exposed to live pupae were less than four eggs per day by day 17, and only one female lived to day 18 of oviposition. This finding represents a significant difference between females exposed to live pupae and females exposed to freezer-stored pupae. Because of the quantities of pupae that must be dissected to determine the fecundity of females exposed to freezer-stored pupae only a limited number were completed during the time period of this study.

Table 5. Fecundity of *Brachymeria ovata* females exposed to four live or freezer-stored *Trichoplusia ni* pupae per day.

B. ovata Reared from:	*B. ovata* Exposed to:	n	# of Eggs/♀ ($\bar{x} \pm$ S.D.)	Range (Eggs)	Mean Longevity
Live Pupae	Live Pupae	14	91.3±51.1a[a]	17-149	14.6a[a]
	Freezer-stored Pupae	4	235.3±316.6b	38-706	60.4b
Freezer-stored Pupae	Live Pupae	13	137.2±59.0ab	22-197	15.6a
	Freezer-stored Pupae	5	422.2±152.4c	157-531	68.5b

[a] Values within a column followed by the same letter are not significantly different ($P > 0.05$, Duncan's multiple range test).

Progeny Production. Progeny production was not significantly impacted when *B. ovata* females were reared from freezer-stored pupae. However, in the first 14 days of oviposition, females exposed to freezer-stored pupae produced about 50 percent fewer progeny than females exposed to live pupae (Table 6). The main cause of this reduction in progeny production is the reduced suitability of freezer-stored pupae for the development of immature *B. ovata* (see Chapter IV). The reduction in the longevity of females exposed to live pupae, again, is probably caused by the presence or lack of a compound in the freezer-stored pupae, as discussed in the longevity and fecundity section.

The mean number of progeny produced per day of oviposition by females reared from live or freezer-stored pupae and exposed to live pupae did not differ significantly during the first seven days of oviposition (Figure 2). On the tenth day of oviposition only one female reared from a freezer-stored pupa was alive, and she produced 13 progeny. The similarity in the number of progeny produced by females reared from live and freezer-stored pupae also was true for the daily progeny production of those exposed to freezer-stored pupae (Figure 3).

During the first three days of oviposition in live pupae, a greater percentage of male progeny was produced (Figure 4). This early emergence of males may help to insure that enough males are present and ready to mate with females when they emerge. Early emergence of males also was observed for progeny produced from freezer-stored pupae. The presence of males when

Table 6. Progeny production of *Brachymeria ovata* during lifetime when exposed to live pupae and during first 14 days of oviposition for those exposed to freezer-stored *Trichoplusia ni* pupae.

Reared from:	Exposed to:	n	Total Progeny (Mean ± S.D.) (Range)	Sex Ratio (♂:♀)
Live Pupae	Live Pupae[a]	14	83.3 ± 26.6a[c] (14-118)	1:1
	Freezer-stored Pupae[b]	14	31.9 ± 15.2b (3-50)	1:1.5
Freezer-stored Pupae	Live Pupae	14	79.1 ± 23.9a (30-119)	1.4:1
	Freezer-stored Pupae	13	37.3 ± 13.0b (10-58)	1.4:1

[a] *B. ovata* females exposed to 30 live pupae per day during their lifetime.
[b] *B. ovata* females exposed to 30 freezer-stored pupae per day for 14 days.
[c] Values within a column followed by the same letter are not significantly different ($P > 0.05$, Duncan's multiple range test).

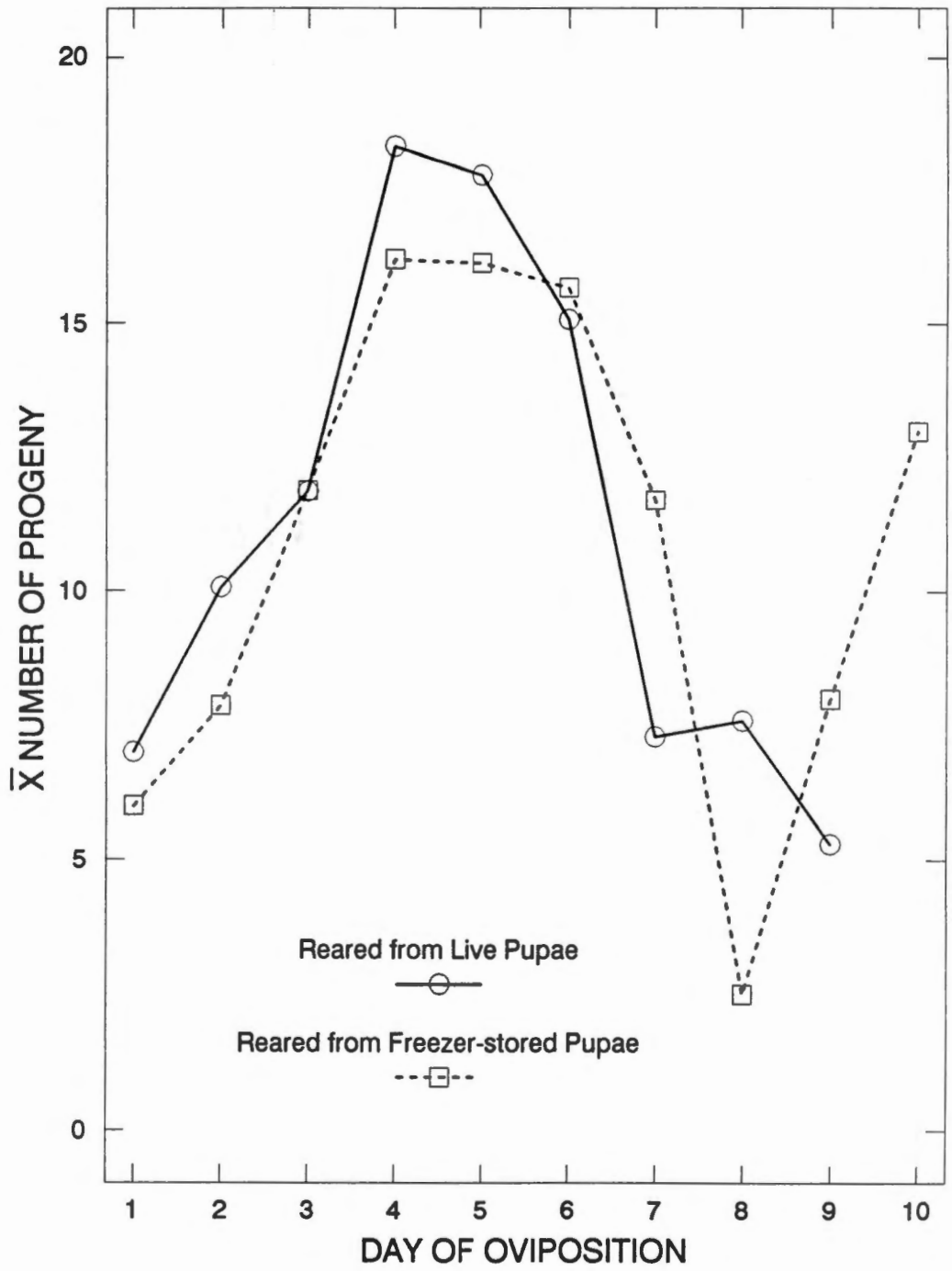

Figure 2. Mean daily progeny production of *Brachymeria ovata* reared from live or freezer-stored pupae and exposed to live pupae during their lifetime.

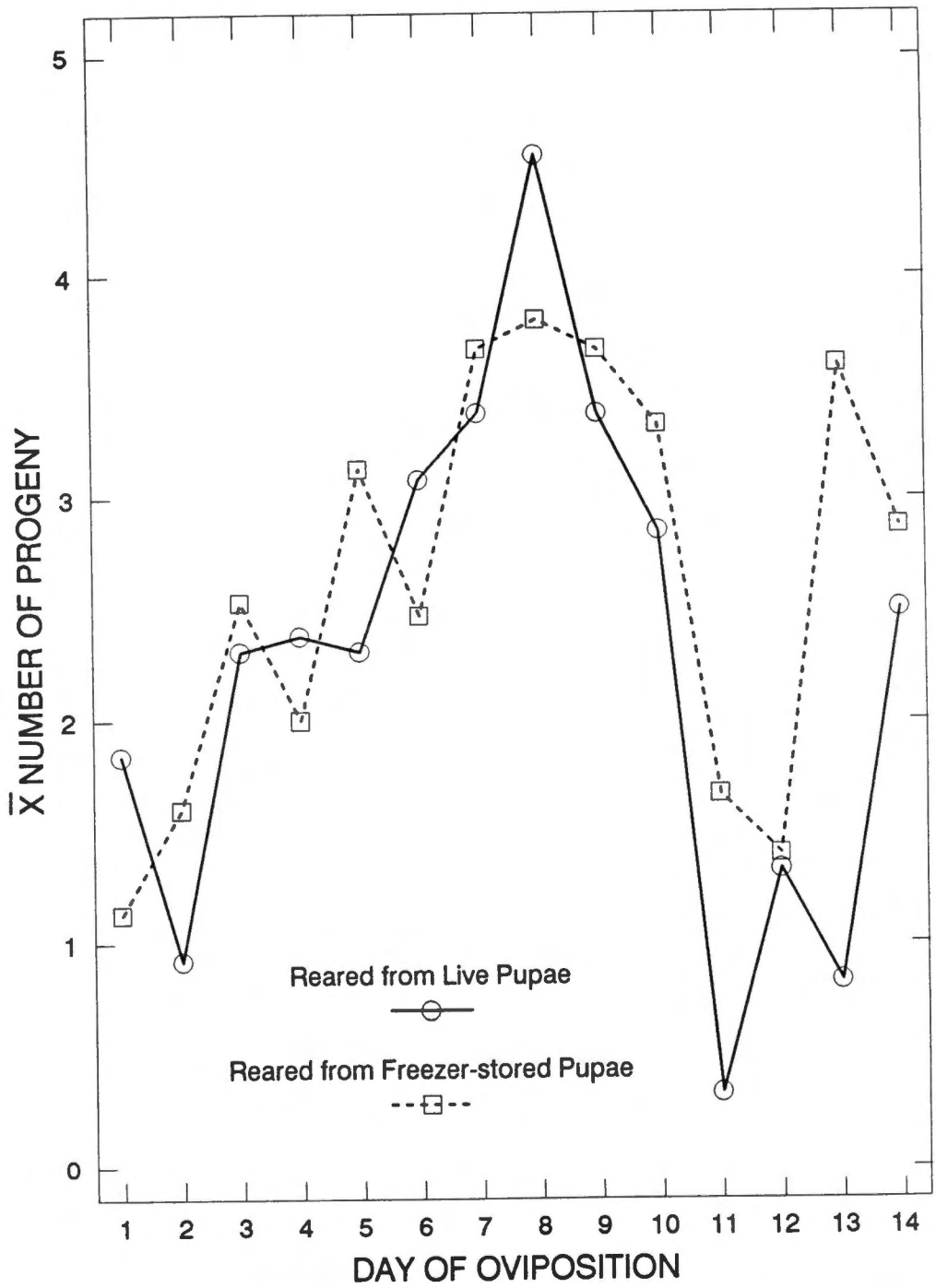

Figure 3. Mean daily progeny production of *Brachymeria ovata* reared from live or freezer-stored pupae and exposed to freezer-stored pupae for the first 14 days of oviposition.

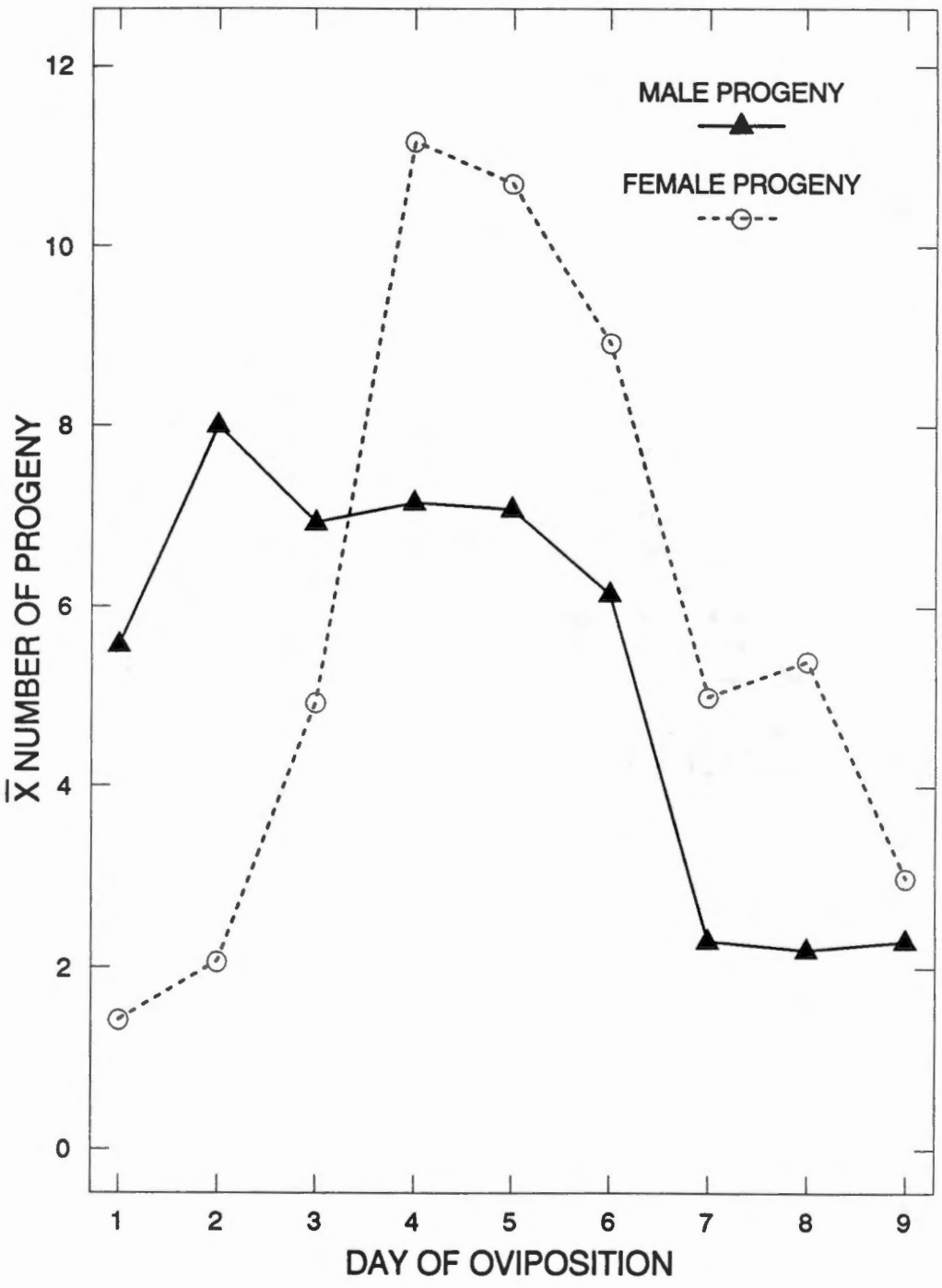

Figure 4. Mean daily male and female progeny produced by *Brachymeria ovata* reared from live pupae and exposed to live pupae.

females emerge has been determined to be advantageous for mate location in some parasitoid species (Askew 1971). In the presence of a newly-emerged female, male *B. ovata* one-day old or more display courtship behavior sooner than newly-emerged males (personal observation).

Data from the fecundity and the progeny production studies for *B. ovata* females reared from live pupae and exposed to live pupae were combined and compared (Figure 5). The curve representing the number of eggs laid follows closely the curve representing the number of progeny produced. This similarity helps to support the validity of the method used to determine fecundity. The increased longevity of females in the fecundity study may be due to their laying fewer eggs during the first six days of oviposition than females in the progeny production study. The number of hosts exposed to females may initially affect the number of eggs laid per day. Females in the fecundity study were exposed to four pupae per day while females in the progeny production study were exposed to 30 pupae per day.

Adult Size. Wing length ranged from 3.34 to 4.67 mm, and tibia length ranged from 0.92 to 1.29 mm. Correlation coefficients were not consistent among replications. The size of these individuals may not have varied enough to have a significant impact upon longevity, fecundity, or progeny production.

Longevity, fecundity and progeny production were not greatly impacted upon by rearing individuals from freezer-stored pupae. However, the type of pupa a female was exposed to when ovipositing significantly affected the

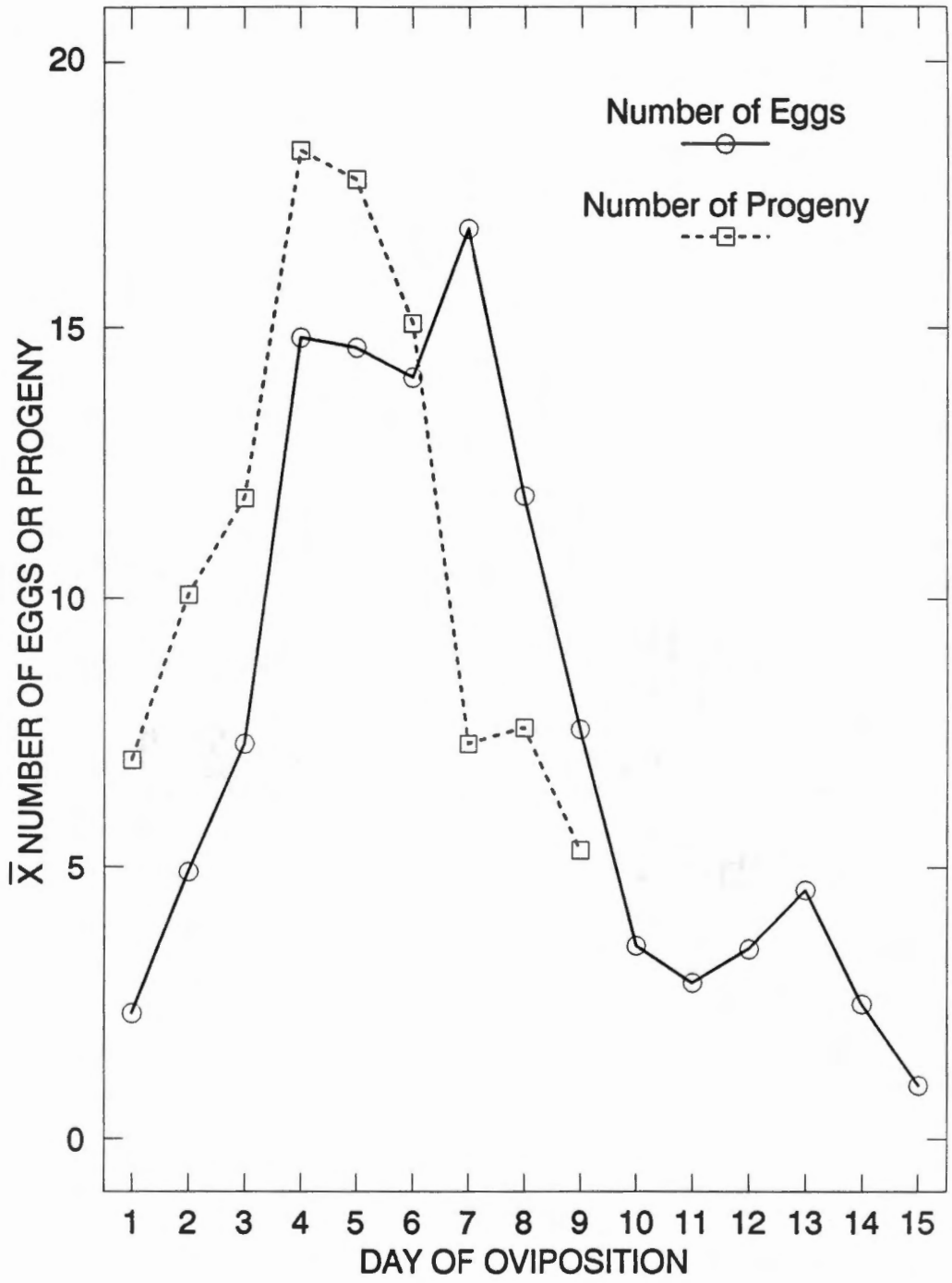

Figure 5. Mean daily fecundity and progeny production of *Brachymeria ovata* reared from live pupae and exposed to live pupae.

previously discussed biological characteristics. Females exposed to live pupae lived a shorter period of time and produced fewer eggs than females exposed to freezer-stored pupae. Results from these studies support the possible existence of a detrimental substance contained within live pupae, or a possible beneficial substance in freezer-stored pupae. In a rearing program utilizing freezer-stored pupae, the possibility exists that females exposed to freezer-stored pupae could produce more progeny than females exposed to live pupae as they can live longer and produce more eggs. To accomplish this increased production, a more suitable freezer-stored host species may need to be used, such as the velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner), which can support up to 88% parasitism of freezer-stored pupae (Grant and Shepard 1987).

Chapter IV

SELECTED BIOLOGICAL CHARACTERISTICS OF *Brachymeria ovata*

Introduction

Parasitoid colonies often must be maintained in the laboratory to provide sufficient numbers of parasitoids for research or for inundative or inoculative releases in the field. The success of a laboratory-reared parasitoid species may be improved if alternate hosts (e.g., multiple species) could be used for colony maintenance. However, the biological traits of the parasitoid species may differ when reared on another host. Thus, when a parasitoid is reared on a new species of host in the laboratory, an evaluation of selected biological characteristics of the progeny should be conducted. Freezer-stored pupae can be considered to be a new type of host for *Brachymeria ovata* (Say) (Hymenoptera: Chalcididae); thus, its impact upon the parasitoid's biology should be investigated.

Previous studies determined that the use of freezer-stored pupae as hosts for rearing *B. ovata* did not adversely affect their reproductive capabilities (Grant and Shepard 1987). They reared the parental generation from freezer-stored pupae and maintained their offspring through the sixth generation. The parasitoids were able to mate and reproduce, but the level of parasitism was not

investigated. Little information, however, is available on the impact of freezer storage on other selected biological characteristics of *B. ovata*.

The objectives of research reported herein were to: 1) determine the acceptability and suitability of freezer-stored pupae, 2) determine the optimal pupal age for freezing, 3) determine the developmental time of the immature stages from freezer-stored pupae, and 4) determine the parasitism rate for the sixth generation of *B. ovata* reared from freezer-stored pupae.

Materials and Methods

The host colony and *B. ovata* colony were maintained as outlined in Chapter II. Experiments were conducted in a laboratory or an incubator maintained at 27±2°C, 50 to 65%RH and a photoperiod of 14:10 (L:D). The cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), was used as hosts in all experiments. Unless otherwise stated, freezer-stored pupae of *T. ni* were obtained by placing live pupae (2 to 4 days old) in petri dishes (100 x 15 mm) in a manual-defrost freezer (maintained at -20°C) for 30 to 90 days. Before exposure to female *B. ovata*, freezer-stored pupae were spread on paper towels and thawed at room temperature for 4 to 6 hours.

Acceptability and Suitability of Freezer-stored Pupae. To determine acceptability and suitability, 25 ovipositing females (4 to 20 days old) reared from live pupae were exposed to 50 live or 50 freezer-stored pupae in a Plexiglas® cage (31 x 31 x 41.3 cm). After 24 hours, 25 pupae of each group

were held in the incubator until adult moth or parasitoid emergence, and the percentage of pupae supporting development of parasitoids to adulthood (i.e., suitability) was determined. The remaining 25 pupae were placed in a freezer (-20°C). These pupae were examined later (30 to 90 days) for ovipositional puncture marks and dissected to determine the number of eggs laid/pupa (i.e., acceptability). The entire experiment was repeated three times.

Optimal Pupal Age for Freezing. The optimal pupal age for freezing was determined by placing 1,050 pupae (1 to 7 days old) in the freezer for 30 to 50 days and then exposing 50 thawed pupae of each age group (i.e., 1, 2, 3, 4, 5, 6, and 7 day-old pupae) to 25 ovipositing female *B. ovata* for 24 hours. Females were chosen randomly from ovipositing females (4 to 30 days old) in the colony by placing one petri dish (100 x 15 mm) containing approximately 50 pupae (2 to 4 days old) in the *B. ovata* colony cage. Twenty-five females that exhibited ovipositional behavior were removed and placed in a separate Plexiglas® cage (31 x 31 x 41.3 cm). After exposure, pupae were held in petri dishes until emergence of adult parasitoids. Live pupae (1 to 6 days old) were exposed to parasitoids in the same manner. As adult moths start to emerge on the seventh day, 7 day old live pupae could not be used. This experiment was repeated three times for a total of 150 pupae for each age group.

Development of Immature Stages in Live and Freezer-stored Pupae. Developmental times for the egg, larval, and pupal stages were determined by exposing 250 live or freezer-stored pupae to the parasitoid colony, containing

approximately 50 to 75 ovipositing females (4 to 30 days old), for 2 hours. On each of the 25 days following exposure, 10 live and 10 freezer-stored pupae were dissected and the immature parasitoids were removed, placed in vials (7 ml), and preserved in 70% ethanol. The number and developmental stage of the parasitoids were recorded for each pupal type. This information was used to determine the developmental times of the larval and pupal stages on live and freezer-stored pupae. The entire experiment was repeated three times.

Percent Parasitism by the F_6 Generation. Six successive generations of *B. ovata* were reared from freezer-stored pupae of *T. ni* (F_6 generation). The percentage of parasitism by the F_6 generation and by females reared from live pupae continuously was compared. One-hundred live pupae were exposed in a Plexiglas® cage (31 x 31 x 41.3 cm) to 25 female *B. ovata* (10 to 30 days old) of the F_6 generation for 24 hours. One-hundred pupae also were exposed, in the same manner as described above, to females that were continuously reared on live pupae. After exposure, pupae were removed, held in petri dishes (100 x 15 mm), and monitored daily for emergence of *B. ovata*. The number of male and female progeny was recorded. This experiment was repeated 3 times.

Data Analysis. Data were analyzed by Student's t test or by analysis of variance and Duncan's (1955) multiple range test where appropriate (SAS Institute 1985). Arcsin transformations of percentages were conducted prior to evaluation. A 0.05 level of probability was used to determine significant differences.

Results and Discussion

Acceptability and Suitability of Freezer-stored Pupae. The acceptability, or the percentage of pupae that contained eggs was significantly less for freezer-stored pupae than for live pupae (Table 7). This reduction may be caused by the attractiveness of live versus freezer-stored pupae, as freezer-stored pupae were determined to be less attractive to female *B. ovata* (Grant and Noblet 1991). However, the mean number of eggs laid per pupa was not significantly different between live or freezer-stored pupae. The suitability, relative to the percentage of pupae producing adult progeny, of freezer-stored pupae was significantly less than that of live pupae. The reduced suitability of freezer-stored pupae may be a result of a decline in the nutritional value of the pupa, or it may be a compound such as a hydrolytic enzyme that may be released during freezer storage and subsequent thawing. Females, on the average, inserted their ovipositors nine to ten times for each egg they laid. A similar phenomenon has been observed for the ichneumonid pupal parasitoid *Itoplectis conquisitor* (Say) whose ovipositor was inserted into pupae five to six times for each egg deposited (Leius 1961).

Optimal Pupal Age for Freezing. The optimal pupal age for freezing was 2 to 5 days after pupation. Many (about 92%) of the freezer-stored one-day-old pupae did not produce adult *B. ovata* and were classified as unsuitable hosts, possibly as a result of incomplete tanning of the cuticle (Table 8). Although older freezer-stored pupae (6 and 7-day-old) and live pupae (6-day-old)

Table 7. Acceptability and suitability of live and freezer-stored *Trichoplusia ni* pupae.

Pupal Type	% Pupae Containing Eggs	Eggs/Pupa ($\bar{x} \pm$ S.D.) (Range)	% Pupae Producing Adult Progeny	Stings/Pupa[a] ($\bar{x} \pm$ S.D.) (Range)
Live	88.2a[b]	4.1±5.1a (0-42)	85.5a	36.7±21.6a (6-99)
Freezer-stored	71.4b	4.2±6.1a (0-35)	23.0b	42.1±25.8a (2-120)

[a] Average number of times an ovipositor was inserted into a pupa (determined by the number of puncture marks on the pupa).
[b] Values within a column followed by the same letter are not significantly different ($P > 0.05$, Duncan's multiple range test); arcsin transformation was performed on percentages before data analysis (n=150).

Table 8. Influence of pupal age on production of *Brachymeria ovata* when reared on live and freezer-stored *Trichoplusia ni* pupae.

Pupal Age (Days)[a]	% Freezer-stored Pupae Producing Progeny	Sex Ratio ♂:♀	% Live Pupae Producing Progeny	Sex Ratio ♂:♀
1	8.1a[b]	4.8:1	68.8a	2.7:1
2	22.2b	2.4:1	68.6a	1.7:1
3	22.9b	2.4:1	80.1a	2.4:1
4	23.2b	4.7:1	72.7a	1.7:1
5	23.5b	8.4:1	75.9a	1.8:1
6	22.9b	12.1:1	76.5a	1.3:1
7	19.5b	7.3:1	------[c]	------

[a] Number of days after pupation [at time of exposure for live pupae or at time of freezing for freezer-stored pupae].
[b] Values within a column followed by the same letter are not significantly different ($P > 0.05$, Duncan's multiple range test); arcsin transformation was performed on percentages before data analysis.
[c] Seven-day-old live pupae were not exposed to *B. ovata*, as adult moths had begun to eclose.

supported development of comparable numbers of *B. ovata*, adult parasitoids were smaller than those reared on two to five day old pupae. A smaller amount of suitable food is present in older pupae because the adult moth has completed much of its development. These reduced food amounts in older pupae may be responsible for the production of smaller *B. ovata*.

Pupae of *T. ni* require about seven days to develop to adulthood and may be successfully parasitized at any age after pupation by *B. ovata*. Studies of the suitability of different age pupae for rearing of the parasitoid *Brachymeria intermedia* (Nees) have determined that pupae of the greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae), 0, 1, and 9 days old produce significantly ($P > 0.05$) fewer adults than pupae 2 to 8 days old (Minot and Leonard 1976b). Live house fly pupae, *Musca domestica* L. (Diptera: Muscidae) were suitable hosts for the pteromalid *Muscidifurax zaraptor* Kogen and Legner at all ages except the first day (Petersen and Matthews 1984). However, when house fly pupae were freeze-killed, they were acceptable at all ages.

Live pupae parasitized by *B. ovata* exhibit a pathology similar to that of eggs parasitized by *Telenomus heliothidis* Ashmead (Hymenoptera: Scelionidae). Pupae parasitized by *B. ovata* cease development within one day of parasitization and cease all movement within three days. Pupae that have been parasitized within one day of their expected emergence as an adult moth will cease development; the parasitoid egg will hatch in about one day and continue to develop to adulthood. Similarly, development in eggs parasitized by *T.*

heliothidis is arrested at nearly all stages of development, except in the pharate larval stage (Strand et al. 1986). The cessation of development in parasitized eggs is due to an arrestment factor injected through the ovipositor along with the egg. As pupal development is arrested quickly, probably before the egg hatches, an arrestment factor also may be associated with parasitism by *B. ovata*.

No significant differences in progeny production were detected among live pupae of different ages when exposed to *B. ovata* (Table 8). Because the pupa survives for about one day after parasitization, the cuticle of live one-day-old pupae may continue to harden fully, allowing them to become suitable hosts for *B. ovata*. Seven-day-old live pupae were not used in this experiment as adult moths had begun to eclose.

The sex ratios (male:female) of *B. ovata* reared on different age live pupae favored males only slightly (ranged from 1.3:1 to 2.7:1) (Table 8). The sex ratios of *B. ovata* reared on different age freezer-stored pupae also favored males. However, these sex ratios varied greatly (2.4:1 to 12.1:1), and may have been male skewed because of the possible use of young or unmated females in this experiment.

Development of Immature Stages in Live and Freezer-stored Pupae. *Brachymeria ovata* reared from freezer-stored pupae require about one day longer to develop to the adult stage than individuals reared from live pupae (Grant and Shepard 1987). The developmental times of the larval and pupal stages were significantly longer when *B. ovata* were reared from freezer-stored

pupae. Developmental times of *B. ovata* larvae and pupae from freezer-stored pupae were extended for as many as five days and one day, respectively, as compared to those reared from live pupae (Figure 6). The period of adult emergence was also extended by one day for freezer-stored pupae. Development of *B. ovata* may be slowed because freezer-stored pupae may contain less suitable food relative to its nutritional value and larvae may have to consume a greater amount of the pupal contents in a freezer-stored pupa than in a live pupa. A *B. ovata* larva usually does not consume the entire contents of a pupa in which it develops (Dowden 1935).

Percent Parasitism by the F_6 Generation. Parasitism of *T. ni* pupae by sixth generation *B. ovata* that had been reared consecutively from freezer-stored pupae was not significantly different when compared to parasitism by females reared only from live pupae. Sixty-four percent of the live pupae exposed to the F_6 generation of *B. ovata* reared from freezer-stored pupae produced adults whereas 81% of live pupae exposed to females reared from live pupae produced adults. Therefore, parasitism of live hosts by *B. ovata* reared from freezer-stored pupae may not be affected if the parasitoids were used in a biological-control program.

In conclusion, these data show that developmental time of *B. ovata* is slowed from freezer-stored pupae, and two to five-day-old pupae are the optimal age for use in freezer storage. While the acceptability of freezer-stored pupae was high, suitability (i.e., progeny production) was low. As high as 67 and 88%

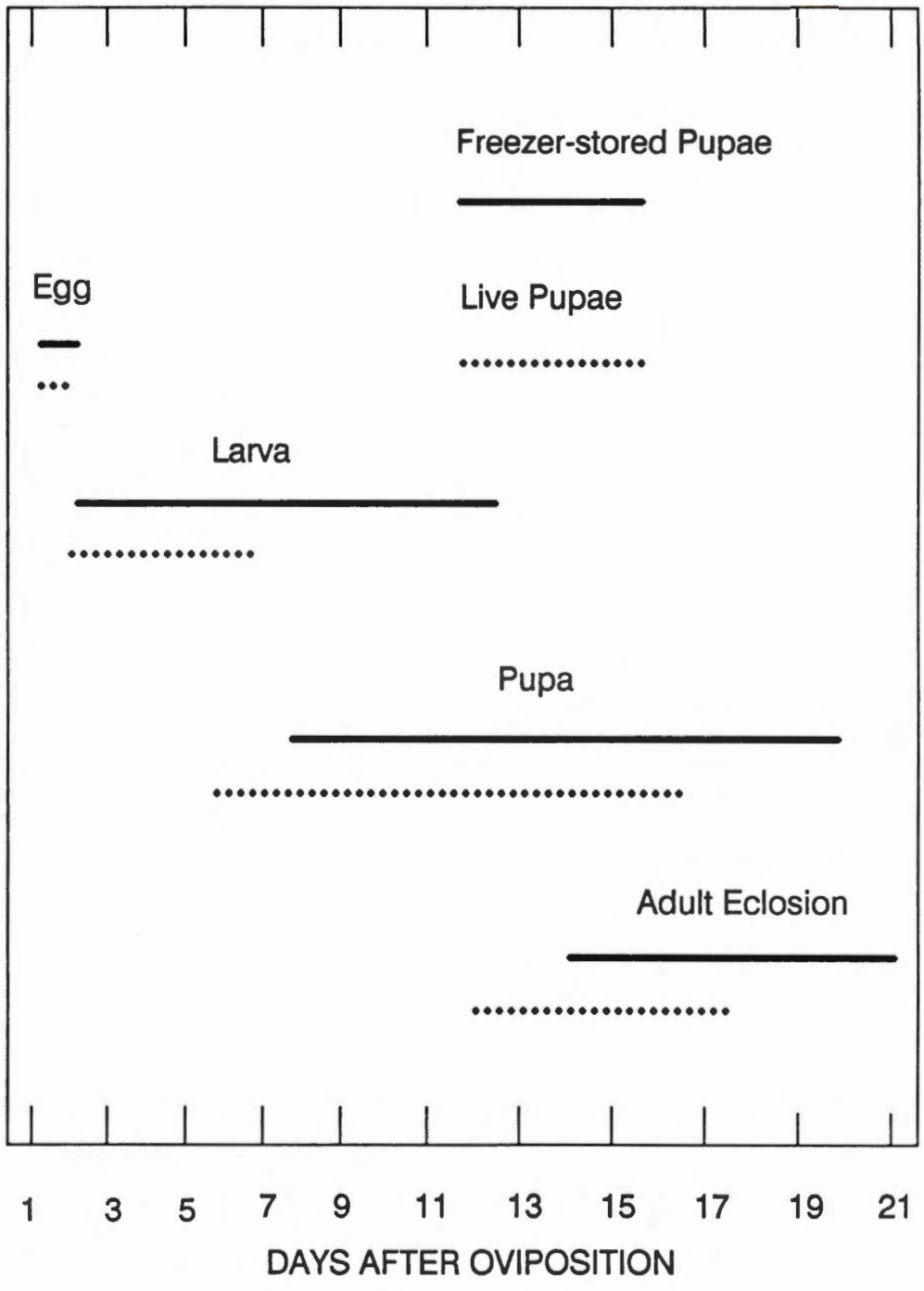

Figure 6. Development of *Brachymeria ovata* reared from live and freezer-stored pupae.

emergence of *B. ovata* adults reared from freezer-stored pupae of *T. ni* and velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner), respectively, has been reported (Grant and Shepard 1987). At these levels, freezer-stored pupae may be desirable and suitable for rearing *B. ovata*. Further research on refinement of freezer storage techniques for maximum production of *B. ovata* is needed. Continuous rearing of *B. ovata* from freezer-stored pupae for use in biological control programs may be possible as the parasitism level of these progeny from live pupae was not reduced significantly.

Chapter V

DISCUSSION

The use of freezer-stored pupae may be a practical means to rear large numbers of *B. ovata*. Data reported herein support this supposition. Overall freezer-stored pupae did not detrimentally impact upon the biological characteristics of *B. ovata*.

The mating ability of individuals reared on freezer-stored pupae was not significantly impacted; however, a slight decrease in successful matings was observed when males were reared from freezer-stored pupae. This reduction, while not statistically significant, could affect the progeny sex ratio resulting in a high percentage of males in a rearing program.

The mean preovipositional period increased when individuals were reared from freezer-stored pupae. This increase may be a result of the overall slowed development of *B. ovata* from freezer-stored pupae. An increase in the preovipositional period should not adversely impact upon the use of freezer-stored pupae in a rearing program or a biological control program.

Brachymeria ovata generally parasitized pupae during the hours of light. The greatest number of parasitoids were produced during the first four-hour period of light and the number of progeny produced decreased throughout the day. The number of pupae killed, which include those parasitized and those

killed by parasitoid-related mortality, did not decline throughout the day. A high level of parasitoid-related mortality would be beneficial in a biological control program. The decrease in the number of progeny produced per four-hour period throughout the day may be a result of the rate at which eggs are produced in the female's ovaries. *B. ovata* females possess two ovaries each with three ovarioles.

Unmated female *B. ovata* individuals not exposed to hosts generally lived longer (114.0 and 135.8 days, when reared from live and freezer-stored pupae, respectively) than unmated males (approximately 100 days). Unmated females reared from freezer-stored pupae lived significantly longer than unmated females reared from live pupae. Paired adults lived for shorter periods than unmated *B. ovata*. Longevity of paired females exposed to live pupae was significantly shorter (approximately 15 days) than the longevity of males or females exposed to live or freezer-stored pupae (approximately 60 to 86 days). Female longevity is decreased with constant exposure to, and oviposition in, hosts. Females reared from freezer-stored pupae may live longer as the result of the lack of a detrimental compound suspected to be present in live pupae. Exposure to live pupae, as opposed to freezer-stored pupae, shortens the longevity of adult females, probably as a result of feeding on pupal fluids and obtaining a detrimental compound. Compounds such as hormones or enzymes in live pupae may decrease *B. ovata* longevity. The suspected detrimental compound also may be a chemical obtained from the larval diet and carried over to the pupal stage.

The longevity of *B. ovata* males decreased as the number of times they mated increased (unpublished data).

Fecundity of *B. ovata* increased when females were exposed to freezer-stored pupae. Those females reared on and exposed to freezer-stored pupae produced the greatest number of eggs ($\overline{x}$=422.2). Females exposed to live pupae lived one fourth to one third as long when reared from live or freezer-stored pupae, respectively, and produced an average of 91.3 and 137.2 eggs, respectively. The decrease in fecundity is related to the decrease in longevity of females exposed to live pupae, which may be a result of a detrimental compound present in live pupae as discussed above.

Progeny production was significantly reduced when females were exposed to freezer-stored pupae. Females produced an average of 83.3 and 79.1 progeny when reared from live or freezer-stored pupae, respectively, and exposed to live pupae during their entire lifetime. Females exposed to freezer-stored pupae produced significantly fewer progeny ($\overline{x}$=31.9 and 37.3, respectively, when reared from live or freezer-stored pupae) during the first 14 days of oviposition. As the fecundity of individuals exposed to freezer-stored pupae is greater than that of ones exposed to live pupae, the potential exists for greater progeny production from females exposed to freezer-stored pupae. The reduced progeny production of females exposed to freezer-stored pupae was primarily the result of the reduced suitability of the freezer-stored pupae to support development of *B. ovata* larvae.

The acceptability of freezer-stored pupae was lower than that of live pupae in terms of the percentage of pupae containing eggs (88.2% of live pupae and 71.4% of freezer-stored pupae) but the average number of eggs per pupa was not significantly different. The suitability of live and freezer-stored pupae in terms of the percentage of pupae supporting development of *B. ovata* to adulthood was significantly higher for live pupae (85.5%) than for freezer-stored pupae (23.0%). Higher levels of suitability were observed in other experiments utilizing freezer-stored pupae. Most parasitoids that do not develop in the freezer-stored pupae die in the larval stage.

The optimal age of pupae for freezing was determined to be from two to five days after pupation. Many individuals reared on older pupae were smaller than those reared on pupae two to five days old, probably as a result of having less food available to the larva. One day old pupae are unsuitable as the integument is not fully hardened, rendering them susceptible to breakage during handling, and desiccation after parasitization. The optimal age for live pupae was also two to five days after pupation.

The development of immature stages of *B. ovata* on freezer-stored pupae was lengthened from two to five days in the larval stage and about one day in the pupal stage. The lengthened developmental time may be the result of the contents of freezer-stored pupae containing less nutritional value than the contents of live pupae.

Rearing *B. ovata* from freezer-stored pupae did not detrimentally impact upon their biological characteristics. An advantage of utilizing freezer-stored pupae is that females exposed to freezer-stored pupae live longer and produce more eggs than females exposed to live pupae. With increased suitability of freezer-stored pupae as observed in some experiments conducted in this study, as well as other studies (Grant and Shepard 1987), it may be economically advantageous to use freezer-stored pupae as hosts for *B. ovata*.

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