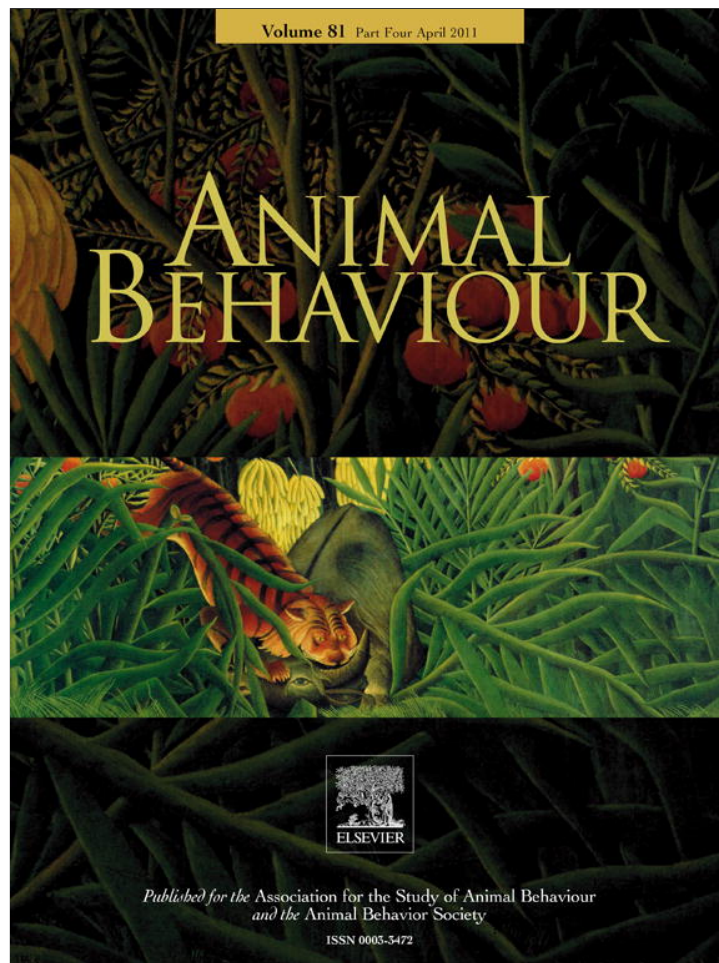


Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

The mating status of mothers and offspring sex affect clutch size in a polyembryonic parasitoid wasp

Na'ama Morag^{a,1}, Amos Bouskila^{a,1}, Olga Rapp^{a,1}, Michal Segoli^{a,b,2}, Tamar Keasar^{c,3}, Ally R. Harari^{a,d,*}

^a Department of Life Sciences, Ben Gurion University

^b Department of Entomology, University of California, Davis

^c Department of Biology, University of Haifa, Oranim

^d Department of Entomology, Agricultural Research Organization, The Volcani Center

ARTICLE INFO

Article history:

Received 15 November 2010

Initial acceptance 8 December 2010

Final acceptance 25 January 2011

Available online 23 February 2011

MS. number: 10-00797R

Keywords:

clone

Copidosoma

epigenetics

heritability

parasitoid wasp

ploidy

Mothers can modify the phenotype of their offspring both genetically and epigenetically, in response to the environment they experience. The role of maternal experience in affecting offspring clutch size is often difficult to measure, because parent–offspring conflict over this trait is common. In polyembryonic parasitoids, where each egg proliferates to form a clone of genetically identical siblings, the conflict is minimized. Therefore, they are good models for studying maternal effects on clutch size. In the haplodiploid parasitoid wasp *Copidosoma koehleri*, female clones contain more individuals than male clones. This may result from differences in genome size or in optimal body size between the sexes. Alternatively, maternal mating may mediate epigenetic physiological changes in eggs, leading to increased proliferation. We evaluated these hypotheses by comparing the body size and number of clone mates produced by mated versus virgin females. Sons of mated females were significantly smaller and formed larger clones than sons of virgins. Daughters formed the largest clones, but resembled sons of mated females in body size. Clone sizes of parents and offspring were not correlated. These findings suggest that both offspring sex and maternal experience (mating status) affect clone size, and that this trait's heritability is low. The increased proliferation by offspring of mated females may enhance paternal fitness by producing extra daughters, or benefit the offspring under conditions where the associated decrease in body size does not greatly reduce fitness. We estimated the contribution of large body size to male reproductive success through a mate choice assay.

© 2011 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Offspring phenotypes are affected by the genes they have received from their parents and the environment they experience, but can also be modified epigenetically in response to maternal environment (Mousseau & Fox 1998a, b). For example, environmental conditions experienced by ovipositing females affect the size of eggs produced, their nutrient composition and the choice of oviposition sites, in numerous invertebrate and vertebrate taxa. These modifications can be based upon the mothers' perception of their living conditions before offspring production, and upon their evaluation of future offspring environment (Bernardo 1996).

Two variables that are likely to be influenced by maternal, nongenetic effects are the number of offspring produced in a clutch and their size (Mousseau & Fox 1998a). In many species, resources for offspring development are limited, and thus a larger clutch may result in a smaller body size per offspring (Godfray 1994). Generally, larger body size is correlated with higher fitness because it increases life span, competitive ability and reproductive success (e.g. Honek 1993; Mayhew & Glaiot 2001, for hymenopteran parasitoids). Thus, optimal clutch size should reflect a balance between the selective benefits of many offspring versus the benefits of large ones (Lack 1947). The relative benefits of large size may depend on environmental conditions (Godfray 1994). Thus, if females can modify clutch size and offspring body size, they should do so in accordance with the conditions they expect their offspring to experience (Mousseau & Fox 1998a, b). Such modifications can be carried out by merely adjusting the number of eggs laid per clutch, but also through active maternal care, for example via transfer of nutrients or hormones to the offspring. At the molecular level, mothers may epigenetically silence some of their offspring's

* Correspondence: A. R. Harari, Department of Entomology, Agricultural Research Organization, The Volcani Center, PO Box 6, Bet Dagan 50250, Israel.

E-mail address: aharari@volcani.agri.gov.il (A.R. Harari).

¹ N. Morag, A. Bouskila and O. Rapp are at the Department of Life Sciences, Ben Gurion University, PO Box 653, Beer Sheva 84105, Israel.

² M. Segoli is at the Department of Entomology, University of California, Davis, CA 95616, U.S.A.

³ T. Keasar is at the Department of Biology, Faculty of Natural Sciences, University of Haifa, Oranim, Tivon 36006, Israel.

genes while activating others, through genome modifications such as methylation (Mousseau & Dingle 1991; Bossdorf et al. 2008).

Studying maternal effects on clutch and body size may be complicated by parent–offspring conflict. Such a conflict is expected, as parental fitness is often maximized at a larger clutch size than is optimal for each descendant (Trivers 1974). Controlling the degree of this conflict can help identify the relative contributions of parents versus offspring to clutch size variation. This can be achieved through the study of polyembryonic species. In this group, a clone of several genetically identical offspring develops from a single egg. Thus, both conflicts among siblings and conflicts between offspring and their parents are expected to be minimal.

Previous studies have investigated the factors that affect clutch size and body size in the wasp *Copidosoma koehleri* (Encyrtidae: Hymenoptera). This polyembryonic parasitoid parasitizes the potato tuber moth, *Phthorimaea operculella* (Gelechiidae: Lepidoptera; Kfir 1981). Each egg divides repeatedly (proliferates) to produce approximately 40 genetically identical embryos, henceforth called a clone (Segoli et al. 2009a). However, clone size is not fixed: it can vary from 20 to 80 individuals (Morag 2009) and correlates negatively with the body size of the clone members (Segoli et al. 2009b). If more than one egg is laid within a host, several clones of varying relatedness may compete for the host resources. In this case, the number of offspring emerging from the host is influenced by the competitive interactions between developing embryos. Competition is mediated by female soldier larvae, which attack both male and female clones that develop within the same host (Segoli et al. 2009b). Dissections along embryonic development suggest that each female clone produces one soldier larva, which is already active before the rest of the larvae differentiate. Male clones, on the other hand, do not produce a soldier (Segoli et al. 2009a). The soldiers' aggression increases as the genetic relatedness between competitors decreases. Consequently, broods of unrelated female clones contain fewer individuals than broods consisting of related clones (Segoli et al. 2009b). These findings point to the combined roles of offspring environment (intraspecific competition) and offspring genetics (relatedness to competitors) in shaping the number and size of offspring. However, they do not address the potential effects of parental genes and environment on these offspring traits.

To address this gap we studied the contributions of hereditary and epigenetic parental effects to phenotypic differences in number and body size between male and female clones in *C. koehleri*. As in other haplodiploid hymenopterans, in *C. koehleri* diploid, fertilized eggs develop into female offspring, while haploid, unfertilized eggs result in male offspring (Cook 1993). When only one *C. koehleri* egg develops within a host, female clones contain significantly more wasps than male clones, and female individuals are accordingly smaller than males (Doutt 1947; Segoli et al. 2009a, b). We first analysed the contribution of parental genetic background to the variation in offspring clone size and body size by calculating regressions for these traits between parents and offspring. Next, we tested whether the differences between male and female clones, regarding the wasps' number and size, may be attributed to the ovipositing mothers' environment. Such an environmental factor can be the mothers' mating status. Evidence for the effects of maternal mating on offspring phenotype includes laying of larger eggs by multiply mated seed beetles, *Callosobruchus maculatus*, compared to singly mated females (Fox 1993); lower reproductive success in sons of multiply mated bulb mites, *Rhizoglyphus robini*, versus sons of singly mated ones (Kozielska et al. 2004); and interactions between maternal mating regime and rearing temperature, which affect offspring fitness (Tregenza et al. 2003). This line of reasoning can be extended to haplodiploid species, in which females that produce daughters are necessarily mated,

whereas both mated and virgin females can produce haploid sons. Differences in clone size may therefore arise between sons and daughters, if maternal mating induces increased offspring proliferation. This maternal environment hypothesis predicts similar clone sizes in sons of mated females and daughters, since both are produced by mated females, and smaller clones of sons produced by virgin females. Owing to the trade-off between clone and body sizes, the sons of virgin females are predicted to be larger than sons and daughters of mated females.

A second hypothesis for intersexual differences in clone and body sizes, which does not preclude the first one, implicates *C. koehleri*'s haplodiploid genetic system (Doutt 1947). According to this view, females owe their extensive proliferation to their diploid genome, whereas haploid males are not capable of proliferating to the same extent. A variant of this argument suggests that differences in optimal body size between males and females may select for different optimal proliferation levels between the sexes. This hypothesis thus stresses the role of offspring genetic make-up in affecting their phenotype. It predicts that clone size and body size would differ between males and females because of their different ploidy or different selection pressures for body size, regardless of their parents' mating experience.

If both offspring genetic make-up and maternal environment additively affect offspring proliferation, then clone sizes are predicted to be largest for females, intermediate for sons of mated females and smallest for sons of virgin females. This is because two factors (maternal mating and a diploid genome) favour larger clone sizes in females. In sons of mated females, only one factor (maternal mating) promotes increased clone sizes, whereas in sons of virgins none of these factors operate. The inverse trend in body size is expected, with sons of virgin females predicted to be the largest and daughters' body size the smallest. We tested these predictions by comparing clone and body sizes among daughters of mated females, sons of mated females and sons of virgin females.

METHODS

Study Species and Rearing Conditions

Copidosoma koehleri females parasitize their host, *P. operculella*, during its egg stage. The host goes through four larval instars after emerging from the egg, while the parasitoid embryos develop inside it. Eventually, the parasitoid larvae consume the host tissues until only its cuticle remains, pupate in the host mummy and emerge as adults. Egg-to-adult development requires ca. 30 days at 27 °C. Adult body length is ca. 1.5 mm. Adult wasps live for about 10 days after emergence and do not provide care to their offspring after egg laying (Keasar et al. 2006). Male-only, female-only and mixed-sex broods occur in natural populations, with frequencies of 0.4, 0.2 and 0.4, respectively (Segoli et al. 2009b). The extent of sib mating, virginity and dispersal under natural conditions is unknown.

A laboratory stock of *C. koehleri* that originated from South Africa was used in the study. The laboratory culture was initiated in 1991, and was supplemented with field-collected wasps in 2005. The wasps were raised according to Berlinger & Lebiush-Mordechi's (1997) protocol. The stock of *P. operculella* was raised at 27 °C, 40% relative humidity and a 12:12 h light:dark schedule. They were fed on potato tubers during larval stages, and on honey and water through adulthood.

Experimental Design

Effects of maternal mating status and genotype on offspring traits

As the F0 generation for the experiment, we used 41 mixed-sex broods (containing males and females that emerged from the same

host) obtained from the insectary stock, 24 h after emergence. *Copidosoma koehleri* females are receptive immediately after emerging as adults (unpublished observation); therefore these 24 h old females were assumed to have mated. One female from each brood was allowed to oviposit five times, each time in a different host. Oviposition was confirmed by direct observation. The single oviposition ensured that the F1 offspring emerging from each of the hosts belonged to one clone only.

As F1 offspring started to emerge, we selected 11 male clones and 11 female clones as parents of the next (F2) generation. To avoid pseudoreplication, clones of the same sex had different mothers. Fifteen females from each clone were allowed to mate, and 10 females per clone remained virgin. Mating partners for females from the same clone were taken from a single male clone, to reduce variability in potential paternal effects. Each pair was placed in a petri dish and observed until mating. Most pairs mated within 1 h of their encounter. Females that did not mate within 8 h were either provided with other mates or were removed from the experiment. Both mated ($N = 165$) and virgin ($N = 110$) females were then allowed to oviposit once in a single host. Hosts were reared until emergence of the wasps of the F2 generation.

Time to parasitoid pupation, the mass of the parasitized host (mummy) at wasp pupation and time to adult emergence were recorded. After emergence, the F2 offspring were sexed and counted. The head width of five individuals per clone was measured in μm , using the software 'AnalySIS', as an indicator of body size (Segoli et al. 2009a, b). Data on head width were averaged for each clone; hence each clone comprises a unit of replication and contributes one data point for body size. In some of the clones, offspring did not complete pupation and did not emerge. Thus, we were not able to measure the head width of these offspring. This reduced sample sizes for the head width data.

Only seven female clones emerged from the parasitized hosts in the F2 generation. We therefore used previous unpublished data on clone and body sizes of 61 additional female clones from single, directly observed ovipositions to learn about the differences between male and female phenotypes. Mothers and fathers of the 61 additional clones were not related to each other. The clones from both data sets had parents from the same insectary stock, and were reared under identical conditions.

Size effects on fitness via reproductive success

The differences in body size between sons of mated and virgin females, if they exist, may affect their fitness by influencing reproductive success. To test whether large body size confers a reproductive benefit, we placed two males of different sizes in a petri dish with a single, unrelated virgin female ($N = 34$) and recorded which male was the first to mate. Males were sorted into large and small by visual inspection before mating; however, their body sizes were measured only after copulation, so that the measuring procedure would not interfere with their mating performance. The female was removed from the dish immediately after her first copulation, and was allowed to oviposit five times, once in each host, 1 h later. The parasitized hosts were reared until offspring emergence and the sex, number and size of the offspring were documented. Reproductive success of males was estimated by their mating success and the number and size of female offspring. We used the sex ratio of the offspring as an indication for the fraction of eggs fertilized by the male, an additional measure of reproductive success.

Data Analysis

Parent–offspring correlations in clone size and body size

Linear regressions were employed to examine the relationships between clone size and head width of parents and their offspring.

The clone size and body size of sons were regressed against those of their mothers, and the clone size and body size of daughters were regressed against the mean of both of their parents' traits and the trait value of each parent separately. Regression was also conducted to test for the effect of mothers' traits on the traits of all offspring combined. Trait heritability was calculated as twice the regression slope (Liu & Smith 2000).

Effects of reproductive status on offspring traits

Body size data for male and female offspring were normally distributed, while clone size data were not (Shapiro–Wilk tests: male body size: $W = 0.94$, $P = 0.13$; female body size: $W = 0.95$, $P = 0.06$; male clone size: $W = 0.92$, $P = 0.02$; female clone size: $W = 0.94$, $P = 0.01$). Differences in number and body size between female and male clones were therefore evaluated using a two-tailed t test, while differences in the number of wasps per clone were tested by a two-tailed Mann–Whitney U test. After the group of males was separated into sons of virgins ($N = 23$) versus sons of mated females ($N = 14$), the small sample sizes did not permit comparisons through parametric tests. Thus, differences between the three groups (daughters, sons of mated females and sons of virgin females) were evaluated using Kruskal–Wallis tests, and each two groups were compared post hoc using Mann–Whitney U tests with Bonferroni corrections for multiple comparisons. The descriptive statistics reported for these comparisons are medians and interquartile ranges. Pearson correlations were used to characterize the relationship between the number of wasps per clone and their body size.

Size effects on male reproductive success

The probability of obtaining the observed numbers of copulations with large and small males, under the null hypothesis of random pairing, was calculated using a binomial test. Two-tailed Mann–Whitney U tests were used to reveal differences in offspring primary sex ratio (i.e. the sex ratio of the eggs laid, inferred from the sex of each of the offspring clones) as well as in their tertiary sex ratio (i.e. the proportion of adult males in all offspring of each female). We also used two-tailed Mann–Whitney U tests to compare the clone size and offspring head width among offspring of large and small males.

RESULTS

Parent–offspring Correlations in Clone Size and Body Size

The regression between offspring and maternal clone sizes was not statistically significant nor were there significant regressions between the sons' or the daughters' clone sizes and maternal clone size when analysed separately or between the clone size of daughters and their fathers' clone size. There was also no significant regression between the mean clone size of both parents and the clone size of daughters (Table 1).

The regression between maternal head width and offspring head width (sons and daughters combined) was significant, but the low r^2 indicates that only about 3% of the variation in offspring head width is explained by the mother's head width. This result was not significant for sons or for daughters, when the data were analysed separately for each sex. The regression between daughters' head widths and their fathers' head widths was not significant either. Similarly, no significant regression between daughters' head width and the mean head width of both parents was found (Table 2).

Effects of Maternal Reproductive Status on Offspring Traits

Only 16% of all ovipositions ($N = 275$) resulted in parasitoid pupation. Offspring of mated females did not differ in pupation

Table 1
Clone size: parameters of the parent–offspring regression

	Offspring of both sexes with mothers	Sons and mothers	Daughter and mothers	Daughters and fathers	Daughters and both parents
Regression equation	$Y = -0.3005X + 48.025$	$Y = -0.3989X + 51.476$	$Y = 2.6486X + 89.308$	$Y = 1.1147X + 2.4516$	$Y = -1.9839X - 41.766$
r^2	0.030	0.067	0.289	0.105	0.200
P	0.258	0.122	0.213	0.482	0.314
N	44	37	7	7	7
Heritability	NS	NS	NS	NS	NS

success from those of virgin females (Fisher's exact test: $P = 0.179$). The primary sex ratio in offspring of mated females was 2:1 in favour of males.

The mean clone size of daughters was significantly larger than the mean clone size of sons ($U = 1462$, $N_{\text{daughters}} = 68$, $N_{\text{sons}} = 44$, $P < 0.001$). After distinguishing between sons of virgin and of mated females, we found significant differences in clone sizes between the three groups of offspring (Kruskal–Wallis: $\chi^2_2 = 36.372$, $P < 0.001$). Post hoc tests revealed fewer sons per clone produced by virgin females compared to male clones produced by mated females ($U = 120$, $P = 0.016$), which were in turn significantly smaller than female clones ($U = 307.5$, $P = 0.007$; Fig. 1a).

Mean head width of daughters ($N = 45$) was significantly smaller than sons' head width ($N = 37$; t test: $t_{80} = -2.134$, $P = 0.036$). After separating the sons' data by maternal mating status, we found a significant difference in head width between the groups (Kruskal–Wallis: $\chi^2_2 = 12.13$, $P = 0.002$). The Mann–Whitney post hoc procedure revealed that head width of sons of

virgin females was significantly larger than that of sons of mated females ($U = 76$, $P = 0.007$) and daughters ($U = 270$, $P = 0.001$); however, the two latter groups did not differ in head width ($U = 275$, $P = 0.476$; Fig. 1b). None the less, the negative correlation between body and clone sizes was maintained in all three treatment groups (Pearson correlation: females: $r_{43} = 0.32$, $P = 0.035$; sons of mated females: $r_{12} = 0.64$, $P = 0.013$; sons of virgin females: $r_{21} = 0.71$, $P < 0.001$). We corrected for the trade-off between clone number and head width by multiplying the number of individuals in each clone by their mean head width. This provides an estimate of the total resources acquired per clone. The product of clone size and head width was highest for females (18625.10 ± 4288.64), intermediate for sons of mated females (16160 ± 4230.99) and lowest for sons of virgin females (12859.23 ± 4776.20). The differences between the three groups were statistically significant (Kruskal–Wallis: $\chi^2_2 = 23.89$, $P < 0.001$).

Host pupal mass did not differ between the three wasp groups (medians and interquartile ranges: 13.2, 12.1–15.4 mg for hosts with females; 14.7, 12.4–16.2 for hosts with sons of mated females; 12.8, 10.5–13.9 for hosts with sons of virgin females; Kruskal–Wallis: $\chi^2_2 = 1.942$, $P = 0.379$). Time from oviposition to parasitoid pupation did not differ significantly between the groups either (23, 18.5–24.5 days for females; 20, 19–21 days for sons of mated females; 22, 21–22 days for sons of virgin females; Kruskal–Wallis: $\chi^2_2 = 4.666$, $P = 0.097$). Time from pupation to adult emergence was also similar among treatments (13.5, 12.5–14 days for females; 13, 12–13 days for sons of mated females; 13.5, 12–14 days for sons of virgin females; Kruskal–Wallis: $\chi^2_2 = 4.678$, $P = 0.78$).

Size Effects on Fitness via Reproductive Success

The head width of the large males we used was 14.6% larger, on average, than that of small males, and the two groups differed significantly from one another (paired t test: $t_{33} = 11.443$, $P < 0.001$). Larger males mated significantly more often: 24 of the females that were introduced to two males mated with the larger individual, and only 10 mated with the smaller one (binomial test: $P = 0.012$). Paternal head width did not significantly influence the number of daughters (linear regression: $Y = 70.388X + 9.35$, $r^2 = 0.222$, $N = 13$, $P = 0.104$) or the daughters' head width ($Y = 0.821X + 0.07$, $r^2 = 0.153$, $N = 8$, $P = 0.337$).

On average, 25.5% of each mother's ovipositions resulted in successful development and emergence of offspring. The primary and the tertiary sex ratios of the offspring were not correlated with the size of the mating male (linear regressions: primary sex ratio: $Y = 0.096X + 0.568$, $r^2 = 0.000$, $N = 24$, $P = 0.973$; tertiary sex ratio: $Y = -0.36X + 0.62$, $r^2 = 0.000$, $N = 24$, $P = 0.99$).

DISCUSSION

Recent research on epigenetic inheritance has highlighted the interplay of parental environment, offspring environment and offspring genotype in shaping phenotypic traits (Wolf et al. 1998).

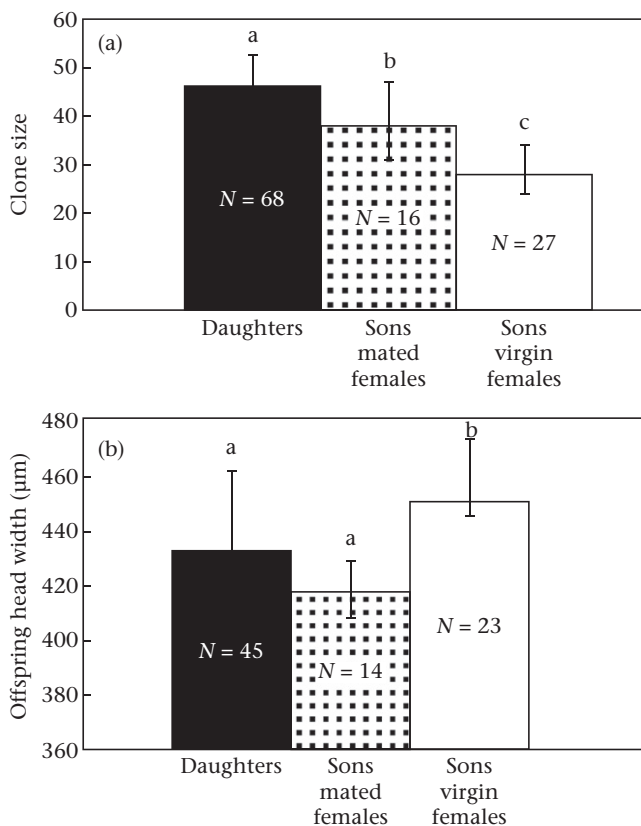


Figure 1. The effects of maternal mating status (mated and virgin females) and offspring sex (daughters and sons) on (a) the number of individuals per clone and (b) the progeny's head width. Medians and interquartile ranges are shown. Different letters above the bars denote significant differences between groups in post hoc tests.

Table 2
Head width: parameters of the parent–offspring regression

	Offspring of both sexes with mothers	Sons and mothers	Daughter and mothers	Daughters and fathers	Daughters and both parents
Regression equation	$Y=0.3525X+426.13$	$Y=0.227X+343.97$	$Y=0.633X+156.2$	$Y=1.111X-95.944$	$Y=1.182X-97.384$
r^2	0.029	0.071	0.107	0.423	0.325
P	0.044	0.117	0.473	0.114	0.181
N	44	36	7	7	7
Heritability	0.70	NS	NS	NS	NS

The heritability estimates in the present study indicate that the genetic effects on clone size are rather weak, whereas head width shows higher correlation between mothers and offspring. Larger samples than used here are probably required to evaluate the heritability of these traits for sons and daughters separately. Nevertheless, both characteristics were also influenced by epigenetic effects, as the reproductive status of females affected their offspring's number and size.

Clone sizes of sons of virgin females were significantly smaller than those of sons of mated females, and both types of male clones were significantly smaller than female clones. These trends remained significant after they were corrected for differences in head width between the three groups. Thus the results suggest that both the mother's reproductive status and offspring's sex affect clone size. The results are therefore compatible with the combined predictions of the 'maternal environment' and of the 'offspring genetic make-up' hypotheses. A survey of six haplodiploid polyembryonic parasitoid species reveals that female clones are larger than male clones in three of the species (*C. koehleri*, *Copidosoma floridanum* and *Phyllocnistis citrella*). In the other three species (*Copidosoma gelechia*, *Copidosoma bakeri* and *Macrocentrus gifuensis*), the opposite trend occurs (Leiby & Raleigh, 1922; Parker 1931; Doutt 1947; Ode & Strand 1995; Zappala & Hoy 2004; Crowley et al. 2009). This suggests that the 'offspring genetic make-up' hypothesis, which suggests effects of offspring ploidy on their proliferation rates (Doutt 1947), cannot solely explain intersexual differences in clone sizes. However, different selection pressures acting on male and female optimal body size, which may vary between species, may account for these observed sex-related differences in clone sizes. For example, differences in the intensity of sexual selection operating on males may affect male size, whereas selection for higher fecundity may affect that of the female (Fairbairn & Preziosi 1994; Fox et al. 2007).

The differences in clone size and head width between the two groups of sons in our experiments support the hypothesis that the females' reproductive status affects their offspring's traits. Alternatively, these differences may be caused by the mere presence of another individual with the female prior to oviposition, regardless of the female's mating experience. This interpretation is less likely, however, because rearing of virgin *C. koehleri* females in groups of 25 individuals for 48 h did not affect the clone or body sizes of their sons (Morag et al., in press). To control fully for the possible effects of male presence on offspring clone size, virgin females would need to be housed with sterile males.

Another possible interpretation is that virgin females are less choosy than mated females when selecting their hosts. They may therefore be more likely to oviposit in suboptimal host eggs, which will ultimately result in smaller clone sizes. We evaluated this possibility using data from two previous studies. The first followed the development of hosts parasitized by mated versus virgin *C. koehleri* females (Segoli et al. 2009a). The proportion of cases in which oviposition was observed but adult moths emerged from the hosts (i.e. possible cases of host rejection) did not depend on the wasps' mating status. The second study scored host rejections of

focal females presented with two previously parasitized hosts. One of the hosts had been parasitized by a virgin *C. koehleri* female, and the other by a mated female (see Segoli et al. 2010 for experimental set-up and the wasps' host choices). Rejection was defined as antennal contact with hosts that did not lead to oviposition within 3 min. Of 32 focal virgin females, 31 rejected one of the hosts and one did not reject any of them. The picture was similar for focal mated females, where 30 of 34 wasps rejected one of the hosts and four rejected none. These observations suggest that mated and virgin females show similar host selectivity; thus hosts parasitized by virgins are not likely to be of lower quality than those parasitized by mated females.

The observed maternal effects can be interpreted as adaptive responses to the environment experienced, or as constraints forced on the ovipositing mothers. According to the adaptive view, virgin females may evaluate their environment as having a low density of males, and therefore anticipate an excess of males in the next generation because of haploid–diploid genetics. Therefore, sons of virgin females are not expected to encounter many mates as adults, unless they disperse to other patches. In several insect species, dispersal abilities increase with body size (e.g. Ellers et al. 1998). According to this reasoning, virgin females may have been selected to produce larger sons in the absence of males, as this could increase their sons' dispersal and/or mating prospects. The production of larger sons can be achieved by producing fewer offspring per clone. Mated females, on the other hand, may perceive their environment as rich in males, and anticipate a high abundance of females in the next generation. Under such conditions, selection for competitive and dispersal abilities among sons is expected to be weaker. Consequently, mated females may be selected to produce larger male clones, as their sons encounter an excess of females and benefit less from large body size than sons of virgin females.

As a partial test of the assumptions underlying this hypothesis, we compared the mating success of larger versus smaller males in a choice situation. The mating assay indeed revealed an advantage to large males over small ones in terms of number of copulations. None the less, mated females did not produce a larger proportion of female clones, even when their mate was large. Moreover, paternal body size did not significantly influence the number and sizes of daughters. These results are congruent with the low father–daughter heritability values recorded for clone and body size, and suggest that the main reproductive advantage of large males lies in increased access to mates. Measuring the effect of body size on fitness via increased dispersal is very difficult in a small insect such as *C. koehleri*, and we did not attempt it in the present study. A positive correlation between body size and longevity was found in a previous experiment (Keasar et al. 2006).

An alternative explanation for the differences between sons of mated versus virgin females is a constraint forced upon the mated females by their mates. Mating elicits major changes in the reproductive behaviour of many insect females, such as increased egg-laying rate and reduced receptivity. In *Drosophila*, these effects are mediated through 'sex peptides' transferred in the male's seminal

fluid during copulation (Chapman et al. 2003; Liu & Kubli 2003). Similarly, we speculate that *C. koehleri* males may transfer signals that enhance offspring proliferation to their partners during mating. These effects may increase a male's fitness when its mate produces daughters, but may also induce proliferation in clones of sons that develop from unfertilized eggs. Thus, the offspring of mated females are expected to be more numerous and consequently smaller than the sons of virgin females, even if the increased proliferation does not optimize female fitness or influence male reproductive success. This provisional interpretation could be tested by searching for analogues of the *Drosophila* 'sex peptides' in the ejaculates of *C. koehleri* males.

To conclude, our study suggests a role for both parental environment and offspring genetics in shaping important phenotypic traits in a polyembryonic parasitoid. One of these traits, body size, also shows considerable heritability. Variation in clone size in response to maternal mating status can be interpreted as an adaptive maternal effect, or as the result of restrictions forced upon the ovipositing mothers by their mates. The effect of offspring genetics, on the other hand, may result from differences in proliferation induced by ploidy. At the ultimate level, they may provide an adaptive adjustment of clone size to achieve optimal body size for males versus females.

Acknowledgments

We thank Zvika Abramsky, Shalhevet Azriel, Yael Lubin, Ittai Malka, Ofer Ovadia, Inon Scharf, and Snir Yehuda for their comments, suggestions and technical help. This study was funded by the Israel Science Foundation (184/06).

References

- Berlinger, M. J. & Lebiush-Mordechi, S. 1997. The potato tuber moth in Israel: a review of its phenology, behavior, methodology, parasites and control. *Trends in Entomology*, **1**, 137–155.
- Bernardo, J. 1996. Maternal effects in animal ecology. *American Zoologist*, **36**, 83–105.
- Bossdorf, O., Richards, C. L. & Pigliucci, M. 2008. Epigenetics for ecologists. *Ecology Letters*, **11**, 106–115.
- Chapman, T., Bangham, J., Vinti, G., Seifried, B., Lung, O., Wolfner, M. F., Smith, H. K. & Partridge, L. 2003. The sex peptide of *Drosophila melanogaster*: female post-mating responses analyzed by using RNA interference. *Proceedings of the National Academy of Sciences, U.S.A.*, **100**, 9923–9928.
- Cook, J. M. 1993. Sex determination in the Hymenoptera: a review of models and evidence. *Heredity*, **71**, 421–435.
- Crowley, P. H., Saeki, Y. & Switzer, P. V. 2009. Evolutionarily stable oviposition and sex ratio in parasitoid wasps with single-sex broods. *Ecological Entomology*, **34**, 163–175.
- Doutt, R. L. 1947. Polyembryony in *Copidosoma koehleri* Blanchard. *American Naturalist*, **81**, 435–453.
- Ellers, J., Van Alphen, J. J. M. & Sevenster, J. G. 1998. A field study of size-fitness relationships in the parasitoid *Asobara tabida*. *Journal of Animal Ecology*, **67**, 318–324.
- Fairbairn, D. J. & Preziosi, R. F. 1994. Sexual selection and the evolution of allometry for sexual size dimorphism in the water strider, *Aquarius remigis*. *American Naturalist*, **144**, 101–118.
- Fox, C. W. 1993. The influence of maternal age and mating frequency on egg size and offspring performance in *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Oecologia*, **96**, 139–146.
- Fox, C. W., Stillwell, R. C. & Moya-Larano, J. 2007. Variation in selection, phenotypic plasticity and the ecology of sexual size dimorphism in two seed-feeding beetles. In: *Sex, Size, and Gender Roles* (Ed. by D. J. Fairbairn, W. U. Blanckenhorn & T. Szekeley), pp. 88–96. New York: Oxford University Press.
- Godfray, H. C. J. 1994. *Parasitoids: Behavioral and Evolutionary Ecology*. Princeton, New Jersey: Princeton University Press.
- Honek, A. 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos*, **66**, 483–492.
- Keasar, T., Segoli, M., Barak, R., Steinberg, S., Giron, D., Strand, M. R., Bouskila, A. & Harari, A. R. 2006. Cost and consequences of superparasitism in the polyembryonic parasitoid *Copidosoma koehleri* (Hymenoptera: Encyrtidae). *Ecological Entomology*, **31**, 277–283.
- Kfir, R. 1981. Fertility of the polyembryonic parasite *Copidosoma koehleri*, effect of humidities on life length and relative abundance as compared with that of *Apanteles subandinus* in potato tuber moth. *Annals of Applied Biology*, **99**, 225–230.
- Kozielska, M., Krzeminska, A. & Radwan, J. 2004. Good genes and the maternal effects of polyandry on offspring reproductive success in the bulb mite. *Proceedings of the Royal Society B*, **271**, 165–170.
- Lack, D. 1947. The significance of clutch size. *Ibis*, **89**, 309–352.
- Leiby, R. W. & Raleigh, N. 1922. The polyembryonic development of *Copidosoma gelechia* with notes on its biology. *Journal of Morphology*, **37**, 195–285.
- Liu, F. H. & Smith, S. M. 2000. Estimating quantitative genetic parameters in haplodiploid organisms. *Heredity*, **85**, 373–382.
- Liu, H. & Kubli, E. 2003. Sex-peptide is the molecular basis of the sperm effect in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences, U.S.A.*, **100**, 9929–9933.
- Mayhew, P. J. & Glazot, O. 2001. Integrating theory of clutch size and body size evolution for parasitoids. *Oikos*, **92**, 372–376.
- Morag, N. 2009. Regulation of offspring's characteristics in the polyembryonic parasitoid wasp *Copidosoma koehleri* via maternal effects. M.Sc. thesis, Ben Gurion University.
- Morag, N., Bouskila, A., Harari, A. & Keasar, T. In press. Trans-generational epigenetic effects of maternal rearing density on offspring development in a parasitoid wasp. *Physiological Entomology*.
- Mousseau, T. A. & Dingle, H. 1991. Maternal effects in insect life histories. *Annual Review of Entomology*, **36**, 511–534.
- Mousseau, T. A. & Fox, C. W. 1998a. The adaptive significance of maternal effects. *Trends in Ecology & Evolution*, **13**, 403–407.
- Mousseau, T. A. & Fox, C. W. 1998b. *Maternal Effects as Adaptations*. Oxford: Oxford University Press.
- Ode, P. J. & Strand, M. R. 1995. Progeny and sex allocation decisions of the polyembryonic wasp *Copidosoma floridanum*. *Journal of Animal Ecology*, **64**, 213–224.
- Parker, H. L. 1931. *Macrocentrus gifuensis* Ashmead, a Polyembryonic Braconid Parasite in the European Corn Borer. Technical Bulletin 230. Washington, DC: United States: Department of Agriculture.
- Segoli, M., Bouskila, A., Harari, A. R. & Keasar, T. 2009a. Developmental patterns in the polyembryonic parasitoid wasp *Copidosoma koehleri*. *Arthropod Structure and Development*, **38**, 84–90.
- Segoli, M., Harari, A. R., Bouskila, A. & Keasar, T. 2009b. Brood size in a polyembryonic parasitoid wasp is affected by relatedness among competing larvae. *Behavioral Ecology*, **20**, 716–767.
- Segoli, M., Keasar, T., Bouskila, A. & Harari, A. 2010. Host choice decisions in the polyembryonic wasp *Copidosoma koehleri* (Hymenoptera: Encyrtidae). *Physiological Entomology*, **35**, 40–45.
- Tregenza, T., Weddell, N., Hosken, D. J. & Ward, P. I. 2003. Maternal effects on offspring depend on female mating pattern and offspring environment in yellow dung flies. *Evolution*, **57**, 297–304.
- Trivers, R. L. 1974. Parent-offspring conflict. *American Zoologist*, **14**, 249–264.
- Wolf, J. B., Brodie, E. D., Cheverud, J. M., Moore, A. J. & Wade, M. J. 1998. Evolutionary consequences of indirect genetic effect. *Trends in Ecology & Evolution*, **13**, 64–69.
- Zappala, L. & Hoy, M. A. 2004. Reproductive strategies and parasitization behavior of *Ageniaspis citricola*, a parasitoid of the citrus leafminer *Phyllocnistis citrella*. *Entomologia Experimentalis et Applicata*, **113**, 135–143.