Sibmating and its Fitness Consequences in the Parasitoid Wasp

Spalangia cameroni (Hymenoptera: Pteromalidae)

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INTRODUCTION

Inbreeding is the mating of close relatives (e.g., sibling-sibling matings or parent-offspring matings). Natural selection for inbreeding avoidance may be common in diploid organisms. With outbreeding, deleterious recessives are commonly in the heterozygous state and hence not expressed. However, with inbreeding the frequency of homozygotes, including homozygous deleterious recessives, increases and fitness is reduced. A reduction in fitness due to inbreeding is referred to as inbreeding depression. Avoidance of inbreeding has been well-documented in some diploid organisms, such as birds and mammals (reviewed in Ralls et al., 1986; Pusey, 1987; Blouin and Blouin, 1988). Mechanisms of inbreeding avoidance include dispersal of one or both sexes prior to mating as well as a reluctance to mate with close relatives.

Haplodiploid organisms may be less prone to inbreeding depression because they may have lower frequencies of deleterious recessive alleles (BrÜckner, 1978; Werren, 1993). Under haplodiploidy, females develop from fertilized eggs and hence are diploid; males develop from unfertilized eggs and hence are haploid. Deleterious recessive alleles are generally exposed to selection when they occur in the haploid sex. However, inbreeding depression may be expressed in diploid females for sex limited traits such as fecundity. Inbreeding depression and inbreeding avoidance have been documented in some haplodiploids (e.g., references in BrÜckner, 1978; Discussion).

Here we test for inbreeding avoidance and inbreeding depression in a haplodiploid organism, the parasitoid wasp Spalangia cameroni. S. cameroni parasitizes the pupal stage of various fly species (Rueda and Axtell, 1985). S. cameroni is described as a solitary species (Rueda and Axtell, 1985), meaning that usually only one offspring completes development on a host. Hosts are sometimes clumped (personal observation), and male and female emergence times overlap (Legner, 1969), making sibmating feasible. Females mate only once whereas males will mate multiply (personal observation). Both males and females can fly, which may reduce the amount of sibmating. Both sibmating and nonsibmating have been documented (Myint and Walter, 1990).

Whether there is any preference for or against siblings has not previously been examined but is examined here, as are fitness effects of sibmating. We measure preference by whether females are more likely to mate with brothers or nonbrothers when given a choice and by duration until mounting and mating occurs

both when females are given a choice and when they are not. We measure fitness effects by the number of adult offspring produced. A reduced number of adult offspring would suggest lower survival. We also examine offspring sex ratio (proportion of sons) because a reduced proportion of daughters would suggest that females are not allowing males to transfer as much sperm or that females are not utilizing sperm as effectively for fertilization.

METHODS

The Spalangia cameroni used in this study were from a colony established in 1985 with wasps that emerged from Musca domestica and Stomoxys calcitrans pupae collected in Indiana, U.S.A (King, 1991). The wasps had been in culture for about 100 generations at the time of the experiments described herein. Voucher specimens are in the insect collection at Purdue University's Department of Entomology. The wasp colony was maintained at 23-28°C, 24 h light using M. domestica as hosts (King, 1988).

In both a no choice experiment and a choice experiment (described below), virgin male-female pairs were placed in test tubes and the duration until the male mounted and the duration until mating began were recorded. Observations were stopped 15 minutes after wasps were placed in the tubes, even if mating had not occurred. Then each mated female was presented with 30 hosts for 24 h in a 1 oz plastic vial with a drop of honey on the side for food. Number and sex of offspring were determined after offspring emerged from hosts as adults.

Hosts were produced following the procedure in King (1988) for large hosts. When presented to the wasps, hosts were less than 24 h old (timed from the initiation of pupal tanning). Each wasp had been isolated in a test tube prior to its emergence so that it had no prior contact with other wasps. On their first day in an experiment, female wasps were 1 or 2 days old; males were 1 or 2 days old, or in the choice experiment rarely 3 days old. Wasps were given a drop of honey if not used when one day old.

In the no choice experiment, females were tested in sister pairs: one of the females was presented with a brother for mating and simultaneously her sister was presented with a nonbrother. Within each sister pair, the sisters were the same age and the males were the same age.

In the choice experiment, each female was placed in a test tube with a brother and a nonbrother. The males were both the same age. The duration until the male mounted and the duration until mating began were recorded. Brothers and nonbrothers were distinguished by marking one with fluorescent powder on the dorsal side of the thorax; which male was marked was alternated between females.

Comparisons were between females that mated with brothers and females that mated with nonbrothers. To compare duration until mounting and mating, rank tests (sign tests or Mann-Whitney U tests) were used so that females that had not mated within the 15 minute observation period could be included and because assumptions of normality were usually not met. Conclusions were the same even when females that had not mated within 15 minutes were excluded from the analyses. T-tests were used for comparisons of mean offspring number and sex ratio when skewness and kurtosis were nonsignificant at alpha of 0.001 (Sokal and Rohlf, 1981). Otherwise, nonparametric tests were used (sign tests or Mann-Whitney U tests).

Two-tailed tests are presented throughout because no specific direction was predicted: theoretical and empirical studies suggest there is avoidance of relatives and hence avoidance of inbreeding depression (e.g., Ralls et al., 1986); however, avoidance of nonrelatives could also be selected for, as a means of avoiding disruption of coadapted gene complexes (Templeton, 1986). Conclusions remain the same if one-tailed tests are used. An alpha of 0.05 was used for comparisons of means. Paired tests (paired t-tests or sign tests) were used in the no choice experiment, with sister-pairs as the sampling unit. Statistical analyses were done using SPSS-PC version 3.1 (Norusis, 1988). Means are presented with ± 1 standard errors.

RESULTS

In the no choice experiment, brothers mounted first as often as nonbrothers (sign test 21 versus 23, P = 0.88) and mated first as often as nonbrothers (sign test 24 versus 24, P = 1.00). Offspring production and proportion of sons did not differ between females that mated with brothers versus nonbrothers (t =

0.06, df = 43, P = 0.95; sign test: proportion of sons greater for nonbrothers in 24 cases, brothers in 13 cases, P = 0.10).

In the choice experiment, females were just as likely to mate with brothers as with nonbrothers (24 versus 20 females, X^2 = 0.36, P > 0.50). As in the no choice experiment, there was no significant difference between brother matings and nonbrother matings in duration until mounting (Mann–Whitney U = 219.0, n_1 = 24, n_2 = 20, P = 0.62) or duration until mating (Mann–Whitney U = 226.0, n_1 = 24, n_2 = 20, P = 0.74). Offspring production and proportion of sons did not differ between females that mated with brothers versus nonbrothers (t = 0.85, df = 40, P = 0.40; Mann–Whitney U = 167.0, n_1 = 19, n_2 = 19, P = 0.69).

In both experiments, the effect of mating with a one day old versus a two day old male was examined among one day old females. Duration until mounting and duration until mating did not differ between females that were with one day old versus two day old males in either the no choice experiment (Mann–Whitney U = 874.5, n_1 = 49, n_2 = 36, P = 0.95; Mann–Whitney U = 987.0, n_1 = 52, n_2 = 38, P = 0.99) or the choice experiment (Mann–Whitney U = 201.0, n_1 = 28, n_2 = 15, P = 0.82; Mann–Whitney U = 200.5, n_1 = 28, n_2 = 15, P = 0.81). Likewise, offspring production and proportion of sons did not differ between females that were with one day old versus two day old males in either the no choice experiment (t = 1.06, df = 85, P = 0.29; Mann–Whitney U = 653.0, n_1 = 44, n_2 = 35, P = 0.25) or the choice experiment (t= 1.21, df = 37, P = 0.24; Mann–Whitney U = 145.0, n_1 = 25, n_2 = 12, P = 0.87).

Duration until mounting and duration until mating did not differ between the no choice and choice experiments (Mann–Whitney U = 1884.5, n_1 = 89, n_2 = 47, P = 0.34; Mann–Whitney U = 2039.0, n_1 = 96, n_2 = 47, P = 0.35). Excluding females that had not mated within 15 minutes (n = 4 in no choice experiment, n = 2 in choice experiment), duration until mounting averaged 159 ± 16 s (n = 131), and duration until mating averaged 162 ± 16 s (n = 137). Number of offspring and proportion of sons also did not differ between experiments (t = 1.08, df = 132, P = 0.28; Mann–Whitney U = 1288.0, n_1 = 83, n_2 = 39, P = 0.07). Number of offspring averaged 13.3 ± 0.5 (n = 134), and proportion of sons averaged 0.22 ± 0.01 (n = 122).

DISCUSSION
There was no preference for or against mating with brothers as evidenced by no significant difference in likelihood of mating with brothers versus nonbrothers in the choice experiment and as evidenced by no difference in duration until mounting or duration until mating in either the choice or no choice experiments. There was no evidence that sibmating affected fitness as measured by number of adult offspring. Offspring sex ratio also was unaffected by sibmating.

Our study suggests that not only does sibmating occur in S. cameroni (Myint and Walter, 1990), but there is no preference for or reluctance towards siblings as mates. In nature, sibmating in S. cameroni will be reduced by males developing more quickly than females (e.g., King, 1988; Myint and Walter, 1990) and both sexes dispersing soon after emergence (Myint and Walter, 1990). That S. cameroni mates with either siblings or nonsiblings is consistent with the absence of a fitness consequence as measured by number and sex ratio of surviving offspring. However, it is still possible that sibmating affects quality, e.g., size, rather than number of offspring.

Whether S. cameroni males exhibit a preference for sisters or nonsisters when given a choice has not been examined. However, male choice seems less likely than female choice given that males mate multiply and given the reputation of male parasitoid wasps for lack of discrimination in mating (Matthews, 1975). Whether there is any avoidance of parent-offspring matings has not been examined. Mother-son matings appear to be uncommon among parasitoid wasps but have been documented in Melittobia. If a female Melittobia fails to mate and hence remains virgin, she will mate with her sons (Browne, 1922; Hobbs and Krunic, 1971).

A limitation of this study is that the wasps used had been in laboratory culture for a considerable time. Thus, brothers and nonbrothers may have been less distinct genetically than would be expected in nature. This is important if kin recognition is based on relatedness per se, rather than on familiarity through a common environment. In the parasitoid wasp Bracon hebetor, females from a strain that had been in culture for more than 60 years distinguished

between sons and brothers that developed on a different host from themselves, though not between such brothers and unrelated males (Petters et al., 1985). The ability to recognize unfamiliar kin has been demonstrated in other laboratory animals (Bateson, 1982; Hepper, 1983; Kareem and Barnard, 1982; Wu et al., 1980).

Whether inbreeding avoidance and inbreeding depression are common in parasitoid wasps is of special interest because of the important role parasitoid wasps have played in studies of sex ratio selection (e.g., Charnov, 1982; King, 1993; Godfray, 1994). Sex ratio theory usually assumes no inbreeding depression (Antolin, 1993). Theory indicates that both inbreeding depression and inbreeding avoidance may select for an increase in the proportion of males (e.g., Hamilton, 1967; Nunney and Luck, 1988; Lively, 1990).

The propensity for and fitness effects of inbreeding have been examined in only a handful of parasitoid wasps. In a review of courtship, Matthew (1975, p. 75) suggests that "matings.. in most species are actually a mixture of many sibmatings and some outcrosses" because "neither sex tends to disperse until after mating." However, the degree of sibmating varies considerably among species (reviewed in Hardy, 1994). Dispersal patterns and preference/avoidance of siblings as mates clearly can contribute to the degree of sibmating, but neither has been well-studied.

Inbreeding avoidance and inbreeding depression have perhaps been best studied in Bracon hebetor. In this species inbreeding avoidance and inbreeding depression are related to the presence of diploid males. Heterozygotes at a "sex locus" are female, hemizygotes are normal haploid males, and homozygotes are diploid males (Whiting, 1943). Inbreeding increases the number of homozygous diploids, i.e., diploid males, but with very low survival to adulthood (only about 5%) (Petters and Mettus, 1980). B. hebetor avoids sibmating, and hence production of diploid males, through dispersal of both sexes from hosts prior to mating readiness (Antolin and Strand, 1992; Ode et al., in press). In the laboratory, only about 14% of B. hebetor females mate before dispersing (Antolin and Strand, 1992). In addition, females avoid mating with males that developed on the same host (Ode et al., in press).

Inbreeding does not result in an increased incidence of diploid males in several pteromalids, Nasonia vitripennis (Skinner and Werren, 1980), Muscidifurax raptor or M. zaraptor (Legner, 1979). Diploid male production has not been examined in S. cameroni (reviewed in Cook, 1993; Godfray, 1994, p. 154). Inbreeding avoidance in N. vitripennis may occur through female preference for males with rare genotypes (Grant et al., 1980).

Inbreeding depression has been documented in haplodiploids not only in relation to diploid males (Brückner, 1978): in honey bees, inbreeding negatively affects workers' wing development, thermoregulation, and recruiting to food sources. Inbreeding depression does not occur, except for interference with sex determination, in the parasitoid wasp Cothonaspis boulardi (Biemont and Bouletreau, 1980). Inbreeding depression has not been demonstrated in the parasitoid wasp Dinarmus vagabundus (Rojas-Rousse et al., 1988).

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