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Timing of oviposition and reproductive skew in cobreeding female burying beetles (*Nicrophorus vespilloides*)

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Burying beetles (genus *Nicrophorus*) are known for their elaborate parental care. Two or more conspecific females may reproduce on the same carcass, especially when the carcass is large. Here we present the results of experiments in which we observed patterns of larval hatching and parental care in unmanipulated cobreeders, manipulated hatching synchrony between cobreeders, and compared patterns of oviposition in cobreeding and single females. Our results show that in these cobreeding associations, one of the females may or may not monopolize the carcass during the period of larval hatching. We present evidence that in either case, infanticide based on temporal cues constitutes an important proximate mechanism underlying the observed reduction in average reproductive success in cobreeding females. Females with higher synchrony (i.e., greater overlap between their oviposition patterns) produce larger broods with lower reproductive skew. Cobreeding females oviposit later and less synchronously than single breeders. Such delayed oviposition may reduce the risk that a female's larvae fall victim to cannibalistic acts committed by her cobreeder or maximize her own opportunity to selectively kill her cobreeder's larvae. *Key words:* burying beetles, cannibalism, communal breeding, infanticide, kin recognition, *Nicrophorus,* parental care, reproductive competition, reproductive skew. *[Behav Ecol 11:357–366 (2000)]*

Reproductive competition is ubiquitous among communally breeding animals. When several conspecific females share a breeding site, as is the case in some vertebrates (''joint nesting'' or ''communal breeding'') as well as in some primitively social or eusocial insects (polygyny), young in the communal nest may compete for food. In this situation, females may increase their individual reproductive success by destroying their cobreeders' eggs or young while sparing their own. Such selective infanticide requires the perpetrator to discriminate between her own offspring and those of cobreeders. One simple mechanism that can enable such discrimination is a temporal switch (Elwood, 1994), sometimes referred to as a ''change in parental state'' (Rosenblatt and Siegel, 1983), which is possibly mediated by hormonal changes triggered by important reproductive events (''state-dependent cues''; Elwood, 1991). Selective infanticide may result if the individual shows infanticidal behavior toward young at a time at which it is likely to encounter unrelated offspring, and parental, non-infanticidal behavior at a time when it is likely to encounter its own (Elwood, 1994). Examples include many bird species with intraspecific brood parasitism or communal breeding, in which females remove eggs from the nest throughout the prelaying period (i.e., until they have laid their first egg) (e.g., Emlen and Wrege, 1986; Macedo and Bianchi, 1997; Møller, 1987; Mumme et al., 1983; Stouffer et al., 1987; Vehrencamp, 1977), and males of many mammalian species that exhibit infanticidal behavior toward the young of females with whom they have not mated or cohabited long enough for the young to be the males' own offspring (see Hrdy and Hausfater, 1984, for examples). Here we report the reproductive consequences of one such temporal switch in cobreeding pairs of female burying beetles *Nicrophorus vespilloides*.

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Natural history of burying beetles

Burying beetles (genus *Nicrophorus*) are known for their elaborate parental care which involves direct interactions between the adult beetles and their developing young (Pukowski, 1933). In *N. vespilloides,* the focus of this study, adults fly in the afternoon and evening in search of the carcasses of small vertebrates (Müller and Eggert, 1987). Upon locating a carcass, single females proceed to bury it without further delay, while lone males typically emit a pheromone that attracts conspecific females (Eggert and Müller, 1989; Pukowski, 1933). Usually, a male and a female participate in the burial of the carcass and the subsequent care of larvae. Females may also perform these activities without the assistance of a male, using sperm stored in their spermatheca to fertilize their eggs (Eggert, 1992). The adults prepare the buried carcass by removing fur or feathers, rolling it up into a ball, and smearing the surface of the carrion with anal excretions (Pukowski, 1933). During this time, the female's ovaries grow rapidly, doubling or tripling their prebreeding mass in 24 h or less (*N. orbicollis*: Trumbo et al., 1995; Wilson and Knollenberg, 1984; *N. vespilloides*: Müller JK, unpublished data). Female *N. vespilloides* lay their eggs scattered in the soil around the carcass. After hatching, the larvae crawl to the carcass; the adults chew a small hole into the carrion ball within which the young reside and where they are fed regurgitated carrion by their parents. The adults defend the carcass and their young against predators or other burying beetles attempting to take over the carcass (Pukowski, 1933; Scott, 1990; Trumbo, 1990a,b).

Conspecific female *N.vespilloides* arriving on the same unburied carcass fight repeatedly and violently. The larger female wins most of these fights (Bartlett and Ashworth, 1988; Müller et al., 1990a; Otronen, 1988) and repels her compet-

itor from the carcass whenever possible, thus severely curtailing access to the carcass by the smaller female (Müller et al., 1990a). In this situation, smaller females typically leave the carcass soon after oviposition and do not provide any care for the young (Eggert and Müller, 1992; Müller et al., 1990a).

Joint breeding in burying beetles

Recently, several studies have documented that interactions between conspecific *Nicrophorus* females are less hostile when the carcass is large for the respective species. Fights are less frequent or absent, and two cobreeders remain with the carcass for more than 5 days (Eggert and Müller, 1992; Scott and Williams, 1993; Trumbo, 1992; Trumbo and Wilson, 1993), well after the time that larvae typically start to hatch and appear on the carcass (Eggert and Müller, 1992). Females are frequently found on the carcass together (Eggert and Müller, 1992; Trumbo, 1992; Trumbo and Wilson, 1993), occasionally feeding the joint brood side-by-side (Eggert and Müller, 1992; Trumbo and Wilson, 1993). Nonetheless, there is evidence for reproductive competition in these apparently peaceful coalitions of females. In a laboratory study of European *N. vespilloides* (Eggert and Müller, 1992) and two field studies of North American *N. defodiens* (Trumbo and Fiore, 1994) and *N. tomentosus* (Scott, 1994), two females cobreeding on large carcasses consistently produced fewer offspring per female than did single females. On medium-sized carcasses, joint broods were often skewed in favor of the larger cobreeder (Müller et al., 1990a; Scott and Williams, 1993), and, likewise, the reduction in reproductive success in the field may have affected the smaller cobreeder exclusively (Scott, 1994). In *N. vespilloides,* however, broods on large carcasses were not significantly skewed in favor of the larger female's offspring (Eggert and Müller, 1992), indicating that body size is not the sole determinant of relative reproductive success on large carcasses.

Based on our earlier work, we hypothesized that females may at least occasionally commit selective infanticide of their cobreeder's offspring. Female *N. vespilloides* reproducing on a carcass by themselves or with a male accept larvae only during a limited window of time, which coincides approximately with the period during which the female's own larvae hatch (''parental phase''). Outside this time window, they cannibalize any larvae they encounter (Müller and Eggert, 1990). When females encounter larvae more than 20 h before their own larvae hatch, they invariably kill these larvae, and some females cannibalize larvae hatching 9–12 h before their own (Müller and Eggert, 1990). Based on these findings, we predicted that an opportunity for temporally based cannibalism of larvae exists if cobreeding females differ significantly in their oviposition times. Here we present the results of laboratory experiments in which we (1) determined the timing of larval hatching and observed the disappearance of larvae in cobreeding pairs of females, (2) manipulated hatching synchrony to determine its effects on various aspects of larval survival and female reproductive success, and (3) compared the oviposition patterns of singly breeding and cobreeding females.

METHODS

Rearing conditions and egg dyes

Experimental beetles were laboratory reared, sexually mature adults aged 20–50 days. Unless otherwise mentioned, they were F_1 offspring of beetles collected in the field at Freiburg, Germany (Mooswald, 48°00′ N, 7°51′E). We kept beetles in small plastic containers half-filled with moist peat and fed them dead mealworms twice a week; up to six same-sex siblings shared the same container. Beetles were kept at 20°C on a 16 h:8 h light:dark cycle. For 2–3 weeks before an experiment, females were fed small amounts of ground beef mixed with a fat-soluble dye. We used either 200 mg Sudan Red 7B or 400 mg Solvent Blue (available from BASF Germany, or Aldrich Chemicals) per 20 g of ground beef. These dyes are incorporated into the eggs at oviposition such that females produce bright pink and blue to turquoise eggs, respectively. This enabled us to identify the eggs of individual females in experiments. Preliminary experiments indicated that the dyes did not affect the timing of oviposition, female fecundity, hatching rate, or larval survival.

General experimental procedure

We kept females with unrelated males for 48 h before the start of experiments to ensure acquisition of a store of viable sperm (see Müller and Eggert, 1989). Immediately before an experiment was initiated, we measured each female's pronotum width under a stereomicroscope equipped with an ocular micrometer. Whenever possible, cobreeding females were matched in size to minimize any size-related differences in oviposition time between beetles, such as have been described for cobreeding female *N. tomentosus* (Scott, 1997). The carcasses used for breeding were large laboratory mice (30 ± 0.3 g) that had been frozen and thawed immediately before an experiment. Experiments were initiated 2 h before the end of the photophase, when beetles are actively searching for carcasses. We first presented females with a carcass in a small transparent plastic box (10 × 10 × 8 cm) for 10–15 min, thereby ensuring that females encountered each other and that they discovered the carcass simultaneously. The carcass, along with both females, was then transferred to a larger transparent plastic box (20 × 20 × 10 cm) half filled with moist peat and returned to the environmental chamber. Upon burial of the carcass, containers were transferred to a dark chamber at 20°C. We checked each container under red light at 12-h intervals to record the location of the two females.

The beetles and their carcass were transferred to a new container with clean peat when they had been on the carcass for 40 h. At this time, we collected the eggs from the old container by searching through small amounts of peat at a time with flexible forceps. Eggs were stored on moist tissue paper in small petri dishes in the same chamber as the beetles to ensure that eggs and females were exposed to similar temperatures, because the duration of embryonic development is highly temperature-dependent (Müller JK, unpublished data). The containers were rotated among the shelves of the environmental chamber every time the beetles or eggs were checked to reduce the effect of slight differences in temperature between boxes that might result from a vertical temperature gradient inside the chamber.

We checked eggs every 4 h beginning 64 h after the start of the experiment, and placed any new larvae on the carcass. To facilitate subsequent observations, the cobreeding females and their carcass were transferred to a clean container with only 2 cm of compacted peat when their first larva had hatched. Before adding larvae, we carefully checked the carcass, turning it with forceps to check for the presence of beetles or larvae underneath, if necessary. We recorded the location of both females relative to the carcass, whether larvae were present, and, if so, which instar(s) they were. Every 48 h, the beetles were again transferred to new containers, and the old ones searched for eggs. We removed females if they had been continuously absent from the carcass for 48 h.

Experiment 1: Observations of unmanipulated cobreeders

The first experiment was designed to determine the oviposition pattern of cobreeding females. Pairs in which one female died ($n = 4$) or laid fewer than five eggs ($n = 1$) were excluded; in addition, we excluded one pair in which less than 30% of one female's eggs hatched, leaving us with 23 of an initial 29 pairs for analysis. The experiment followed the general experimental procedure described above. The maximum difference in pronotum width between cobreeders was 0.2 mm, or 2.9%. We marked females individually by piercing their elytra with insect pins (000) in number-encoding patterns, and for speedier identification during trials, the elytra of cobreeders were marked, one with black, the other with white paint. Two color strains are established in our laboratory: light beetles without any black band in the posterior half of the elytra and dark ones with a black band that covers the posterior half of the elytra. When males from these laboratory strains are paired with wild-type females, their offspring are easily distinguishable (see also Müller and Eggert, 1989), which enabled us to ascertain the maternity of surviving larvae: In each pair of cobreeders, one of the females had been inseminated by a male from one color strain and the other female by a male from the other strain. All surviving offspring could be clearly attributed to one or the other of the females.

Experiment 2: Synchrony and brood sharing in manipulated pairs

In experiment 2, we assessed the effects of experimentally manipulated hatching synchrony on larval survival and various other features of the surviving brood. Synchrony was manipulated by exchanging partners between cobreeding females so as to generate pairs with high synchrony (i.e., those whose larvae hatched more or less synchronously), and pairs with low synchrony (i.e., those whose larvae hatched at different times). Females were paired with their final partners when the larvae of at least one female had started to hatch. Because we were unable to anticipate each female's oviposition pattern, we paired females whose larvae started to hatch within 12 h of each other to generate synchronous pairs, and females in which the onset of hatching differed by 24 h or more to generate asynchronous pairs. In our analyses, we used the continuous variable ''oviposition synchrony'' (see explanation of variables) instead of the two treatment groups because it is a much more accurate descriptor of the females' relative oviposition behavior.

Re-pairing cobreeders was possible because cobreeding females typically do not recognize their partners individually. Trumbo and Wilson (1993) observed that cobreeding *N. defodiens* violently attacked nonbreeding females but not their own nest mates. A study in our laboratory during which female *N. vespilloides* were observed for 15 min after re-pairing demonstrated similar attacks against nonbreeding replacement females but revealed the absence of such aggressive interactions when replacements were taken from other cobreeding associations (Beck, 1995). The re-pairing allowed us to generate systematic variation in hatching synchrony that was independent of the early interactions of the original cobreeding pairs because females were exchanged before the first larvae were placed on the carcass.

In this experiment, we used females from the two color strains that had been inseminated by males of their own color strain (see above). Whenever possible, we re-paired females with partners from the other strain, which allowed us to establish the maternity of surviving larvae. All surviving offspring could be clearly attributed to one or the other of the eventual cobreeders, except in five cases in which we had paired females from the same strain. The experiment followed the same general experimental procedure as described above. Two separate series of observations were initiated with 22 and 30 pairs of females. For our analyses, we pooled data from both series for higher statistical power because we found no significant effect of series or the interactions between series and synchrony on any of the dependent variables (two-way ANOVA, $p > .3$ in all instances). In the analysis of total brood size, we used data from 40 pairs (in 12 pairs, 1 of the cobreeders had died, or laid no eggs, or all her eggs were unfertilized). In the analysis of brood sharing, another 5 pairs could not be used because they consisted of females of the same color, which precluded an analysis of maternity, leaving a sample size of 35 pairs for analyses of brood sharing. Despite the re-pairing, body size differences between final cobreeders were relatively small, with only 5 out of the 40 pairs differing by more than 12% in their pronotum width (median difference = 4.9%).

Experiment 3: Onset and synchrony of oviposition in single breeders and cobreeders

Experiment 3 was designed to assess oviposition strategies of cobreeding females compared to single females. As in experiment 1, we used female F_1 offspring of field-collected beetles, this time inseminated by unrelated males from the same group. The experimental design was as described above, except that three experimental treatments were established. Treatment 1 consisted of females paired individually with males on carcasses (single breeders, $n = 40$). If synchrony between cobreeders simply results from interindividual variation in oviposition time regardless of the presence or absence of another breeder, oviposition synchrony should be the same for cobreeders as for random pairs (dyads) of separately breeding females. To assess the degree of synchrony in the separately breeding females, each single breeder was randomly assigned another single breeder within this treatment, yielding a total of 20 dyads. Treatment 2 consisted of pairs of females cobreeding on the same carcass (cobreeders, $n = 20$ pairs). Treatment 3 consisted of cobreeding groups each made up of two cobreeding females and a male (cobreeders with males, $n = 20$ groups).

Females in cobreeding pairs differed in pronotum width by a median of 1.4% (treatment 2) and 3.0% (treatment 3). As before, they had ingested different colored dyes before being placed on the carcass, and the same was true for each of the randomly assigned dyads of single breeders. Larvae were added to their mother's carcass until a brood was established. We recorded the onset of larval hatching for each individual, as well as the degree of synchrony for each pair of females, including dyads of single breeders. In some replicates, one of the females died or failed to lay any eggs, leaving a total of 18 dyads of single breeders, 17 pairs of cobreeders, and 17 groups of cobreeding females with males.

Definition of variables

Several variables used in our analyses require some elaboration. The oviposition differential of two cobreeders is the difference in the time at which each female's larvae begin to hatch. For this variable and for the time to oviposition in experiment 3, we used the time at which a female's first three larvae had hatched rather than her first larva because single eggs are occasionally laid much earlier than the remaining clutch. We first determined the interval (in multiples of 4 h) from the time at which we observed one female's third larva to the time at which we recorded her cobreeder's third larva. For any observed 4-h difference, the true difference could be

anywhere from 1 to 7 h. If actual differences occur at the same frequency, 5/8 of these should occur between 1 and 4 h, and the remaining 3/8 should fall between 5 and 7 h; the latter differences would more appropriately be assigned to the 5- to 8-h interval. We estimated the actual hatching interval between two clutches from the distribution of observed intervals by assigning 3/8 of the original observations for each interval to the next higher 4-h interval.

Oviposition synchrony was defined as the area of overlap between the two females' relative hatching curves. First we determined the proportion of each female's offspring that hatched in each 4-h interval. If $p_{1,i}$, $p_{2,i}$ are the proportions of female 1's and female 2's larvae that hatch in interval i, respectively, then synchrony (S) is defined as the sum of the smaller of the two values over all intervals i:

$$S = \sum_{i=1}^{n} \min(p_{1,i}, p_{2,i}).$$

If the two female's hatching curves overlap perfectly, S = 1; if they do not overlap at all, S = 0. Oviposition differential and oviposition synchrony are accurately derived from the hatching times of larvae because the duration of embryonic development at 20°C exhibits minimal variation (mean = 56 h, SD = 1.5h, n = 138; Müller, 1987).

Brood sharing describes how evenly the surviving brood is shared genetically between two cobreeding females. If p_1 and p_2 are the proportions of the surviving brood that are the offspring of female 1 and female 2, respectively, then $p_1 + p_2 = 1$. We define brood sharing as $BS = p_1{*}p_2{*}4 = p_1{*}(1 - p_1){*}4 = 4p_1 - 4p_1^2$. Multiplying by four standardizes this measure to vary between one (when both females contribute to the surviving brood equally) and zero (when only one of them leaves offspring). This measure of reproductive partitioning in a group of two breeders equals one minus the index of reproductive skew developed by Reeve and Keller (Keller and Vargo, 1993; Reeve and Ratnieks, 1993).

Brood sharing is a variable that assesses the number of each female's larvae that survive without considering how many of each female's larvae initially arrived on the carcass. In a hypothetical brood in which all larvae survive but one of the females produces a larger number of first-instar larvae, BS < 1 despite the fact that all larvae survive. As a measure of survival that is independent of the number of larvae initially produced, we define the index of shared survival as $SU = s_1{*}s_2$, in which s_1 and s_2 are the survival rates of first-instar larvae produced by females 1 and 2, respectively, calculated as the number of a female's offspring surviving divided by the number of this female's first-instar larvae placed on the carcass. Like our brood sharing index, this index varies between zero and one.

To describe differential fecundity between females, or rather, differences in the numbers of their first-instar larvae that arrive on the carcass, we use an index similar to brood sharing. We define egg sharing as $ES = e_1{*}e_2{*}4$, e_1 and e_2 being the proportions of all first-instar larvae produced by female 1 and 2, respectively; the constant (4) again standardizes the measure to vary between zero and one.

RESULTS

Observations of unmanipulated cobreeders: experiment 1

Oviposition differentials and synchrony

To assess the opportunity for temporally based infanticide, we determined the oviposition differential and synchrony (for definitions, see Methods) of cobreeding pairs. The oviposition differential was frequently quite long (median = 26.5 h, first quartile = 8.5 h, third quartile = 50 h, n = 23, see Figure 1).

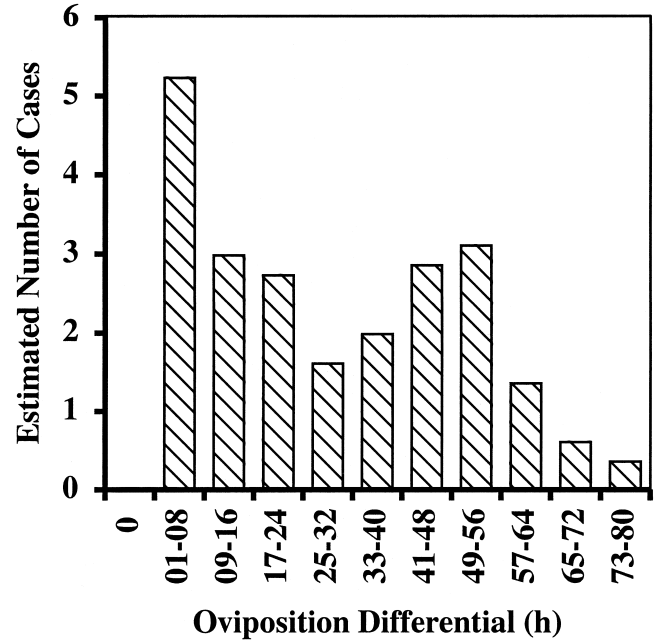

Figure 1
Frequency distribution of oviposition differentials (estimated differences in the time to oviposition) between two cobreeders. For explanation of deviations from integers, see Methods.

We estimated that five pairs started ovipositing within 8 h of each other, whereas in the remaining 18 pairs the hatching differential was longer than 8 h. Based on earlier observations of single females (Müller and Eggert, 1990), this should have been sufficient to allow for some temporally based cannibalism. The distribution of oviposition synchrony did not deviate significantly from normality (Shapiro-Wilk W = 0.930, p = .11, n = 23). Synchrony values were well below 1 in all pairs, with a mean of 0.20 (SD = 0.15, minimum = 0, maximum = 0.51, n = 23).

Female presence on the carcass and disappearance of larvae

We made no systematic attempt to observe acts of infanticide directly, but on two separate occasions, we saw females consume larvae newly arriving on a carcass. Neither did we attempt to follow up on the fate of individual larvae, but our visual inspections at 4-h intervals frequently revealed that larvae placed on carcasses had disappeared 4 h later.

With respect to the cobreeders' presence on the carcass, we observed two discretely different situations, which we describe as exclusive and nonexclusive pairs. In our cobreeding associations, larvae hatched over a median period of 20 intervals (first quartile = 16.25, third quartile = 25.75 intervals, n = 23). In exclusive pairs, one of the females (the "noncaring" female) was seen on the carcass during no more than two inspections during the period of larval hatching, indicating that the other female (the "caring" female) had excluded her and monopolized the carcass. Nonexclusive pairs, on the other hand, were those in which both females were found on the carcass for a minimum of five different observations during larval hatching. Intermediate values of three or four observations on the carcass were conspicuously absent. Severe injuries, involving the loss of tibiae, femora, and entire legs were rare but did occur in four exclusive and six nonexclusive pairs.

Exclusive pairs constituted about half of our sample (12/23). In 10 of these cases, live larvae were first observed on the carcass in the interval immediately following the one in which the caring female's own first larvae had hatched and had been

Table 1

Observed disappearance of larvae from carcasses when carcasses are monopolized by one female (exclusive pairs, $n = 12$) and when both females are on the carcass while larvae are hatching (nonexclusive pairs, $n = 11$)

Time at which larvae were added	Larvae disappeared		
	n Instances (% of pairs)	*n* Larvae range (median)	*n* Intervals range (median)
Exclusive pairs			
Before caring female's larvae start to hatch	4 (33)	2–22 (6)	2–8 (4.5)
After caring female's larvae finish hatching	4 (33)	7–11 (10)	2–7 (5.5)
Nonexclusive pairs			
Before the second female's larvae hatch	11 (100)	1–17 (8)	1–8 (4)
After both females' larvae have started to hatch	11 (100)	6–58 (25)	1–9 (5)

added to the carcass, indicating that the female started to accept larvae at exactly the time her own larvae had begun to hatch. In the two remaining cases, the caring female's own first larvae disappeared, but the larvae added 4 h later did not. In four cases larvae of the noncaring female that had hatched earlier than the caring female's disappeared from the carcass. In each of these cases, all early larvae disappeared, involving several batches of larvae that had been added at different intervals (Table 1). The disappearance of larvae after the caring female had begun to accept and feed larvae was evident only if larvae were added at a time when all the larvae present on the carcass had already molted into second instars; this requires a gap of at least 16 h. We observed the disappearance of larvae that appeared on the carcass too late to be the caring female's offspring in four pairs (see Table 1); in two of these cases, the noncaring female left no surviving offspring. There were no instances in which both early and late larvae disappeared from the same carcass. Survival rates were higher for the caring female's larvae (range 19–100%, median = 70%) than for the noncaring female's larvae (range 0–89%, median = 19%; Wilcoxon matched-pairs signed-rank test, $p = .025$).

In nonexclusive pairs, we frequently noted the disappearance of larvae as well. However, in this situation the disappearance did not simply coincide with the time at which one or both female's larvae started to hatch. In all of these pairs larvae still disappeared after the time at which both females' larvae had started to hatch (Table 1). In two instances, larvae disappeared after they had apparently been accepted (i.e., live larvae had been found in the crater on the carcass during one or several inspections). In all of the pairs we observed, more larvae disappeared after both females' larvae had started to hatch than when only one female's larvae had begun to hatch. We did not observe the disappearance of late larvae in any of these pairs. Overall survival rates of larvae in this group (24–43%, median = 33%, $n = 11$) were significantly lower than in exclusive pairs (39–62%, median = 53.5%, $n = 12$; Mann-Whitney $U = 130$, $p < .0001$). In some pairs, continued infanticide led to the extinction of both females' entire first clutches, forcing them to produce replacement clutches. Oviposition frequently continued (with interruptions) over 4 or 5 days; in one pair, larvae continued to hatch over a period of 7 days.

Synchrony and partitioning of reproductive success

In unmanipulated cobreeders, the degree of oviposition synchrony was significantly correlated with brood sharing ($r_s = .571$, $p = .007$, $n = 23$) but not with the index of shared survival ($r_s = .310$, $p = .146$, $n = 23$).

Differential fecundity as a potential factor affecting brood sharing

In addition to oviposition synchrony, initial differences in the number of eggs laid, or the number of first-instar larvae produced, could also affect the degree of brood sharing in our pairs. To assess the potential impact of such factors on brood sharing, we determined partial rank correlations between egg sharing, synchrony, and brood sharing. There was no significant correlation between egg sharing and brood sharing when synchrony was held constant ($r_{s\ \mathrm{partial}} = .392$, $p = .064$, $n = 23$), no significant correlation between egg sharing and synchrony when brood sharing was held constant ($r_{s\ \mathrm{partial}} = .121$, $p > .5$, $n = 23$), but the correlation between synchrony and brood sharing remained highly significant when egg sharing was held constant ($r_{s\ \mathrm{partial}} = .569$, $p = .008$, $n = 23$).

In exclusive pairs, access to the carcass appeared to affect differential fecundity but not the timing of oviposition: caring females laid more eggs (mean = 35, SE = 3.0, $n = 12$) than their noncaring partners (mean = 24, SE = 3.3, $n = 12$; paired t test: $t = 2.22$, $p = .048$) but did not oviposit earlier (time to oviposition in caring females: mean = 55.0 h, SE = 10.9 h, $n = 12$, in noncaring females: mean = 60.7 h, SE = 8.2 h, $n = 12$; paired t test: $t = 0.582$, $p = .573$).

Manipulations of synchrony: experiment 2

Synchrony and reproductive success of pairs

This experiment was designed to manipulate synchrony between cobreeders so as to generate especially synchronous or especially asynchronous pairs and to assess their subsequent reproductive success as individuals and as a group. The majority of re-paired associations of cobreeding females were nonexclusive (33/40), with the remaining pairs following the "exclusive" pattern described above. As in the previous experiment, many of the larvae added to the carcass disappeared. In several nonexclusive pairs (15/33), entire broods vanished after they had apparently been accepted and larvae had been present on the carcass for three or more consecutive inspections.

Due to our attempts to create pairs with high and low synchrony, synchrony values showed significant deviations from normality (Shapiro-Wilk $W = 0.917$, $n = 40$, $p = .007$), and thus the following correlations are nonparametric Spearman rank correlations. Synchrony of re-paired cobreeders varied between 0 and 0.82, with a median of 0.31 ($n = 40$). Synchrony was significantly correlated with shared survival (Figure 2a: $r_s = 0.763$, $p < .0001$, $n = 35$), brood sharing (Figure 2b: $r_s = .741$, $p < .0001$, $n = 35$), and the total number of offspring produced (Figure 2c: $r_s = .656$, $p < .0001$, $n = 40$). Thus, when females were re-paired with other females whose timing of oviposition was similar to their own, both females'

larvae experienced improved survival rates compared to females that were re-paired with asynchronous partners. More synchronous pairs were also more productive, and they partitioned reproduction more evenly (i.e., reproductive skew was lower).

Differential fecundity as a potential factor affecting brood sharing

As in our first experiment, we determined partial rank correlations between egg sharing, synchrony, and brood sharing. There was no significant correlation between egg sharing and brood sharing when synchrony was held constant ($r_{s\ \text{partial}} = -.028$, $p > .7$, $n = 35$), and no significant correlation between egg sharing and synchrony when brood sharing was held constant ($r_{s\ \text{partial}} = .013$, $p > .9$, $n = 35$), but the correlation between synchrony and brood sharing remained highly significant when egg sharing was held constant ($r_{s\ \text{partial}} = .741$, $p < .0001$, $n = 35$). These results indicate that the effects of the differential production of first-instar larvae on brood sharing, or reproductive skew, were negligible.

Comparing oviposition in single breeders and cobreeders: experiment 3

The previous results indicate that conflicts between cobreeders would be minimized, and overall reproductive success maximized, if females oviposited synchronously. If the behavior of cobreeding females is designed to reduce conflicts or maximize total reproductive success, they should oviposit more synchronously than random combinations of single breeders. On the other hand, if the behavior of cobreeders is designed to maximize individual opportunities for selective infanticide, females should postpone oviposition because delayed oviposition may enable them to recognize and selectively destroy their cobreeder's offspring.

The observed times to oviposition showed significant deviations from normality (Shapiro-Wilk test, all $p < .05$), but synchrony values did not (all $p > .10$). Treatment had a significant effect on the time to oviposition (Kruskal-Wallis test, $H_2 = 36.64$, $p < .0001$): two cobreeding females initiated oviposition later than did single breeders (see Figure 3a). When only the first-laying of the two females in a pair or dyad was considered, this effect persisted (Kruskal-Wallis test, $H_2 = 19.93$, $p < .0001$; Figure 3b). Females in cobreeding pairs clearly did not oviposit more synchronously than randomly assigned pairs of single breeders on the same carcass size, and if anything, they appeared to oviposit less synchronously (Table 2). The presence or absence of a male appeared to have no effect on the timing of oviposition.

DISCUSSION

Disappearance of larvae and infanticide

Many of the first-instar larvae that arrived on large carcasses with cobreeding female *N. vespilloides* disappeared before larvae were finally accepted and cared for. We consider the disappearance of early larvae to be indicative of cannibalism by adults. *N. vespilloides* larvae that are accepted aggregate in a small crater created by the parents in the carrion ball and are thus readily visible. Larvae can survive without parental feeding (Eggert et al., 1998), and therefore a lack of parental care cannot explain their rapid disappearance. Females that are not ready to accept larvae were observed to cannibalize larvae in an earlier study (Müller and Eggert, 1990) as well as in the current one. A female cannibalizing a larva picks it up from the carrion ball and lifts it up in her mandibles, raising her head and thorax while lowering her abdomen in a rearing-up posture we have not observed in other contexts. The larva is then crushed between the female's mandibles and consumed. However, we are not certain that females likewise selectively killed late larvae arriving on the carcass after a brood had

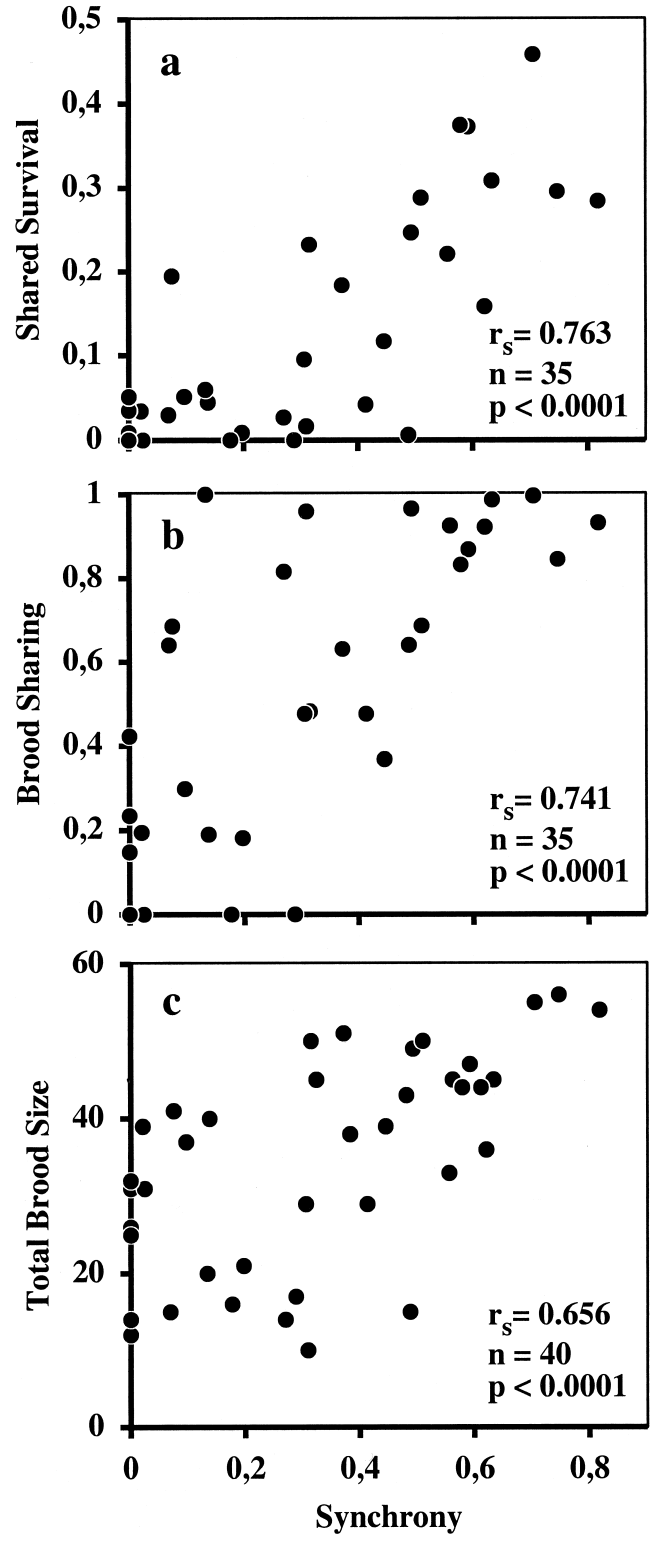

Figure 2
Relationships between oviposition synchrony and (a) shared survival, (b) brood sharing, and (c) total brood size and the results of Spearman rank correlations.

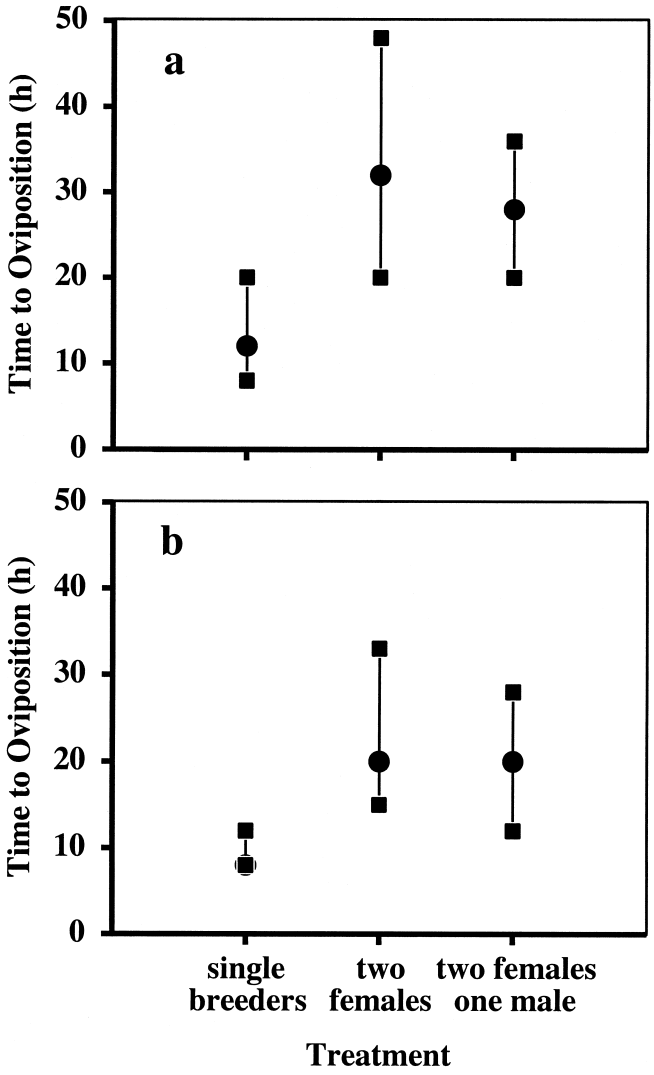

Figure 3
Time to oviposition (median and interquartile range) in single breeders (females breeding with a male), two cobreeding females, and two cobreeding females with a male. (a) All females, (b) the first of the two females in a pair to lay eggs. Single breeders differed significantly from each of the other groups (Tukey-Kramer procedure for ranks, all $p < .01$); the other differences were nonsignificant (all $p > .05$).

already been established. Late-arriving larvae may be hard to see among their larger siblings, and sibling competition and the miry condition of the carcass interior may contribute to their demise even in the absence of adult intervention.

Parental phases in burying beetles

Parental phases, or parental windows of time, outside of which adults cannibalize young coming to the carcass, are likely to occur in both sexes of all burying beetle species. During the parental phase, there is no evidence of any discrimination against nonkin; unrelated conspecific larvae are accepted and fed (Müller and Eggert, 1990; Trumbo and Wilson, 1993), as are larvae of other species. For example, *N. vespilloides* parents can be used to rear larvae of *N. vespillo, N. humator, N. defodiens, N. pustulatus, N. orbicollis,* and even larvae of a Japanese species in the sister genus of *Nicrophorus, Ptomascopus* (Müller and Eggert, 1990; Müller JK, unpublished data). Trumbo (1994) found that *N. orbicollis* females accepted heterospecific larvae added during the parental phase, and that they are susceptible to interspecific parasitism in natural broods of the former. Female *N. defodiens, N. sayi,* and *N. marginatus* all reject larvae arriving on the carcass at the wrong time (Trumbo ST, personal communication), as do male *N. vespilloides* and *N. orbicollis* (Müller and Eggert, 1990; Müller JK, Eggert A-K, unpublished data; Scott MP, personal communication).

Exclusive and nonexclusive cobreeders

Our observations revealed the existence of exclusive cobreeders even on the large (30-g) carcasses, suggesting that noncaring females were denied access to the carcass by caring females. Some of the noncaring females might have chosen to abandon the carcass after oviposition if they had been given the opportunity. However, severe injuries indicate that aggressive interactions must have occurred even in nonexclusive associations. We do not understand all the factors that affect the exclusive or nonexclusive nature of the relationship between cobreeders, but carcass size clearly plays a role. On medium-sized (15-g) carcasses, exclusive interactions between female *N. vespilloides* appear to be the rule (Müller et al., 1990a). The smaller female is continually attacked by the larger female and leaves the carcass after only a few days with little direct access to the carcass, but some of her offspring are reared with the larger female's brood (''intraspecific brood parasitism''). Associations on large (30-g) carcasses are frequently nonexclusive: Both females frequently feed young, sometimes side-by-side (''joint breeding''; Eggert and Müller, 1992), as do females of *N. defodiens, N. orbicollis,* and *N. tomentosus* on carcasses that are large (''communal breeding''; Trumbo, 1992; ''cooperative associations''; Trumbo and Wilson, 1993). The females' relative body sizes may also affect their relationship because a large size discrepancy may facilitate monopolization of the carcass by the larger female. A role for absolute body size is suggested by interspecific differences in the tendency to exhibit communal breeding on carcasses of a given size (Trumbo, 1992; Trumbo and Wilson, 1993).

Switching from infanticidal to parental behavior in cobreeding pairs

The timing of the switch from infanticidal to parental behavior in female *N. vespilloides* is clearly affected by the social situation on the carcass. In the absence of other females, this switch occurs well before the female's own larvae hatch. The time at which these females accept larvae varies from 20–17 h to 8–5 h before their own larvae hatch (Müller and Eggert,

Table 2
Degree of oviposition synchrony in randomly assigned pairs of monogamous females and in two female cobreeders with or without a male

	Single breeders (paired)	Two females (cobreeders)	Two females, one male (cobreeders)
Mean ± SE (n)	0.43 ± 0.05 (18)	0.25 ± 0.04 (17)	0.30 ± 0.05 (17)

ANOVA, $F_{2,49} = 3.417$, $p = .0408$; only the difference between single breeders and two females is significant: Bonferroni/Dunn, $p = .014$.

1990). When another female has detected the same carcass but can be denied access to the carcass, the switch seems to move to a slightly later point in time. In such exclusive or brood parasitic pairs, the caring or host female has the opportunity to selectively destroy larvae coming to the carcass at the wrong time. In this situation, with its greater threat of competition from another female's larvae, females start to accept larvae closer to the exact time their own larvae hatch, occasionally killing some of their own first larvae. When another female has continued access to the carcass, the switch is further delayed, frequently well into the period during which each female's own larvae hatch. In most nonexclusive pairs, larvae were not allowed to survive until long after the time at which both females' larvae started to hatch. Being unable to discriminate against unrelated offspring at this time, females must frequently kill their own larvae along with those of their cobreeder's. If the onset and continuation of parental care is regulated by hormones such as juvenile hormone, as has been suggested by Trumbo (1997), the hormone level itself or its action on the respective neurons must be modified by cues associated with the initial, or continued, presence of another female on the carcass.

Synchrony and reproductive success

When cobreeding pairs oviposit more synchronously, larger proportions of both females' larvae survive, reproduction is partitioned more evenly, and overall productivity increases. We contend that this is due to reduced opportunities for temporally based selective infanticide of unrelated larvae under conditions of synchrony, regardless of the type of association between females (exclusive or nonexclusive). The relative timing of oviposition, and, concomitantly, larval hatching, plays an important role in determining the reproductive skew and the reproductive output of the pair. Little is known about the factors that determine the time at which a female begins to oviposit or her specific oviposition pattern. The female's physiological condition at the time she detects the carcass may play a role, and our data indicate that social factors, such as the presence of a conspecific female, may have an effect as well. We think that synchrony is likely to be a more or less accidental and unpredictable consequence of both female's oviposition times because the two females likely have no reliable information about each other's oviposition pattern. However, the observed delay in oviposition relative to single breeders suggests that the cobreeding female *N. vespilloides* do not behave in ways that minimize conflict or maximize group reproductive output. Instead, the observable behavior of cobreeding females likely results from each female's selfish attempts to increase her own representation in the surviving brood.

Timing of oviposition in cobreeders: an evolutionary game?

Despite the fact that we have little knowledge about possible causes of variation in female oviposition behavior, we suggest that oviposition in cobreeding females might be viewed as an evolutionary game in which females can choose the onset of oviposition and the length of the period over which they oviposit but are limited in the number of eggs they can produce in a certain time interval. Physiological constraints appear to limit the number of eggs produced before hatching of the first larva in several *Nicrophorus* species (Müller et al., 1990b; Trumbo, 1992). Females could oviposit as soon as possible after carcass detection, as late as the carcass remains usable as larval food, or at any time in between these extremes. Larvae hatching early would be able to utilize the carcass while it is in optimal condition, but they would be highly vulnerable to infanticide by a later-laying female. Larvae from late clutches, while relatively safe against infanticide, would have to cope with a carcass of significantly reduced value. Spreading oviposition over a long period of time virtually ensures that some offspring will survive, but it also entails an exceptionally high risk of accepting the cobreeder's larvae. This tactic is vulnerable to partners that lay their eggs in a short amount of time and are then able to selectively kill earlier and later larvae. Shortening the oviposition period affords the female a fairly precise means of identifying her own larvae but entails a high risk of complete loss of this clutch. A formal model might prove more fruitful than these considerations, which do not reveal an optimal oviposition tactic.

Other factors affecting reproductive skew

Reproductive skew, or brood sharing, describes the final result of a joint breeding attempt, which can be determined by different mechanisms. In burying beetles, differential reproductive success of cobreeders can be influenced by a whole host of factors (e.g., see Eggert and Müller, 1997). Effects of the breeding association (one or several females) on patterns of oviposition and parental care as revealed in the present study were largely ignored in earlier attempts to explain patterns of joint care in burying beetles (Eggert and Müller, 1992; Scott, 1994, 1997; Trumbo and Wilson, 1993), leading authors to believe that cobreeders oviposit more or less synchronously and that infanticide, if it occurs at all, is restricted to brief, early periods of a reproductive attempt (Eggert and Müller, 1992; Scott, 1994; Trumbo and Wilson, 1993). Indeed, Scott (1996, 1997) ruled out any role for selective infanticide in the reproductive skew observed in cobreeding *N. tomentosus,* attributing it instead to early behavioral interactions, differential fecundity, and selective oophagy.

In the present study, factors affecting the number of first-instar larvae on a carcass, such as differential oophagy or differential fecundity, may have had some effect on final reproductive skew, but such effects were weak compared to the overwhelming impact of oviposition synchrony. Our experimental design should have provided ample opportunity for selective ovicide, but currently, we have no reason to assume that cobreeding *N. vespilloides* exhibit this behavior. Even in *N. tomentosus,* evidence for oophagy is indirect, and underlying mechanisms remain unclear (Scott, 1996, 1997). Species differences are likely because breeding associations of *N. tomentosus* may differ from *N. vespilloides* in other respects as well: Females begin laying soon after they have detected the carcass, both females finish laying within 48 h after burial, and hatching is restricted to a period of about 24 h (Scott, 1997).

We suspect that body size differences might have played a larger role had our cobreeders been less closely matched in size. Size-related fecundity differences have been demonstrated in *N. vespilloides* (Bartlett and Ashworth, 1988), and the outcome of aggressive interactions becomes highly predictable when body size differences are more substantial (Müller et al., 1990a). Müller et al. (1990a) and Trumbo et al. (1995) suggested that dominance-related differential access to the carcass may have quantitative effects on clutch size, an idea supported by the larger clutch sizes for caring females in exclusive breeding associations in our first experiment. It is likely that such differential access exacerbates size-related fecundity differences. In *N. tomentosus,* reduced access to the carcass also seems to delay the onset of oviposition (Scott, 1997), but the present study found no evidence of such a delay in *N. vespilloides.*

Consequences of infanticide: counter-adaptations and communal breeding

Both larvae and adults may have developed counter-adaptations to larval cannibalism, which occurs in cobreeding females (this paper), during or after takeovers (Trumbo, 1990a,b), and in the context of brood reduction (Bartlett, 1987). During time periods in which no surviving larvae were observed on the carcass, we occasionally observed a few first-instar larvae off the carcass, moving around in the soil. We think that such larvae may have escaped cannibalism by temporarily abandoning the carcass. In one instance, our maternity data demonstrated the survival of several larvae that we had not seen during one visual inspection. These larvae may have been concealed inside the carcass, which could have provided some protection against infanticide. The most important counter-adaptation for cobreeders, however, may be their delayed oviposition. Such a delay likely entails significant costs due to the deterioration of carcass quality and the consumption of carrion by adults. Overall, cobreeding appears to be associated with significant costs to females. Cobreeders still incur injuries, they may have to produce very large numbers of eggs to compensate at least partially for the losses due to cannibalism, and they may eventually produce fewer offspring because of low oviposition synchrony. In view of the fact that potential benefits of communal breeding would have to be quite large to outweigh these costs, we maintain that breeding associations are not preferable to females, but are merely a consequence of the females' inability to monopolize large carcasses. We suggest that the opportunity to bias the composition of the surviving brood through larval infanticide constitutes a major incentive for a second female to remain on the carcass.

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