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Resource presence and operational sex ratio as determinants of copulation duration in the fly *Rhagoletis juglandis*

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The effects of the interaction between the operational sex ratio (OSR) and a resource (i.e. oviposition site) on mating dynamics have rarely been considered. We examined the effect of the resource presence and its interaction, with the effect of OSR on copulation duration in *Rhagoletis juglandis*, a tephritid fly species characterized by a resource-defence mating system in which males defend territories on walnut fruit. In this species, copulation duration varies from 30 s to over 1 h and was shown previously to respond strongly to changes in OSR. In the field, short copulations tended to begin and end on fruit, whereas most long copulations generally began on fruit but ended in the foliage, suggesting a possible effect of resource presence on the copulation duration. In laboratory assays of isolated pairs, copulations were significantly shorter in the presence of a surrogate fruit, confirming the effect of resource presence. In another laboratory assay, in which we manipulated OSR independently of resource presence, resource presence and OSR were additive in their effects. Results are discussed in the context of sperm competition theory.

Both operational sex ratio (OSR) and the distribution of essential resources are known to influence the dynamics of mating traits (Emlen & Oring 1977). A significant body of literature has addressed the effect of OSR on mating traits such as copulation duration and male harassment (Lawrence 1986; Arnqvist 1992; Krupa & Sih 1993; Souroukis & Cade 1993; Vincent et al. 1994; Weatherhead et al. 1995). In most species, longer copulations predominate in male-biased environments, whereas shorter copulations predominate in female-biased ones (McLain 1981; Sillén-Tullberg 1981; Clark 1988). These effects are consistent with sperm competition theory. In male-biased environments, the likelihood that a mated male will encounter another female is relatively low. Thus, males should invest relatively more sperm per mating, and consequently, copulations should be longer; furthermore, in male-biased environments, the female with which a male is currently mating is likely to mate again, and males could again compensate by copulating longer and transferring relatively more sperm (Thornhill 1980; Dickinson 1986; Wolf et al. 1989). Sperm transfer aside, long copulations may reflect a kind of mate guarding, which may benefit males in male-biased environments (Thornhill & Alcock 1983).

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A separate body of literature has addressed how control by males of resources critical to survival or reproduction mediates access to females (Parker 1971, 1972; Emlen & Oring 1977; Siva-Jothy 1987). In particular, the location of male–female encounters with respect to the resource is known to influence mating traits, including copulation duration. In dragonflies and dung flies, for example, males copulate for shorter periods at oviposition sites than they do elsewhere in the habitat (Parker 1971; Siva-Jothy 1987; Ward & Simmons 1991). In some species, the rate of encounters between males and receptive females has been observed to be relatively higher within a territory than away from it (Parker 1970, 1978; Siva-Jothy 1987). The potential for additional copulations away from the resource is relatively low and thus males mating off the resource are expected to invest relatively more sperm in (and thus engage in longer copulations with) a given female. Short copulations at the resource might benefit the female as well, if they provide a female with relatively better opportunities to use the resource.

Although OSR and resource presence presumably act simultaneously in nature to influence mating dynamics, their joint effects have seldom been evaluated in a systematic way. Our own observations of a tephritid fruit fly, *Rhagoletis juglandis*, provided special incentive for evaluating the joint effects of OSR and resource presence. In a previous study (Alonso-Pimentel & Papaj 1996a), OSR

exerted a strong effect on copulation duration in *R. juglandis* both under laboratory and field conditions. A male-biased sex ratio was associated with a predominance of long matings, whereas a female-biased sex ratio was associated with a predominance of short matings. In the field, copulations generally begin on fruit (where OSR is typically male biased) but, owing to harassment by other males, frequently move to host foliage (where OSR is apparently less male biased). We wondered therefore whether the effect of OSR in nature was mediated wholly or in part by an effect of resource presence on copulation duration.

This study had three objectives: first, to quantify the distribution of long and short copulations in the field in relation to a pair's position on fruit versus foliage; second, to determine experimentally whether manipulation of the presence of the resource influences copulation duration; and third, to evaluate a possible interaction between OSR and resource presence in their effects on copulation duration. Based on sperm competition theory, we predicted that the presence of fruit and female-biased sex ratios would be associated with shorter copulations. As a null hypothesis, we further predicted that any effect of the presence of fruit would be independent of an effect of the operational sex ratio.

METHODS

Natural History

Rhagoletis juglandis is a member of the *R. suavis* group, a group of species that infest walnuts (Boyce 1934; Bush 1966). In southern Arizona, this species uses Arizona walnut, *Juglans major*, which can be found in montane canyons between 1200 and 2700 m in elevation. At lower elevations, adults emerge around the middle of June, and at higher elevations, adult emergence starts a few weeks later. Flies remain on a given tree for 4–5 weeks (personal observation). *Rhagoletis juglandis* is characterized by resource defence in which males defend fruit, and mate with females that arrive at fruit to lay eggs. Defence of fruit is characterized by wing displays, chases and boxing matches (Papaj 1994). Copulation duration in *R. juglandis* is highly variable, ranging from 30 s to over 1 h, and is distributed bimodally, being either short (<200 s) or long (>600 s) (Alonso-Pimentel & Papaj 1996a). In this paper, we refer to these two categories as 'short' or 'long' matings, respectively. Copulations in each mode virtually always involve the transfer of sperm.

Animals Used

The flies used in these experiments originated from fruit collected in 1994 and 1995 in Garden Canyon in the Huachuca Mountains in southern Arizona. Flies were reared in a 3.79-litre plastic containers with water, sugar and slips of a protein hydrolysate and sugar mixture in a room with a 14:10 h light:dark cycle and a day temperature of 28°C. Flies used in all the experiments were 13–23 days old, posteclosion. At this age, both males and females are sexually mature. We attempted to randomize the age of the flies used in each experiment by mixing flies that were 13–23 days old. Our previous work indicated that receptivity and copulation duration did not vary significantly between virgin and mated flies (Alonso-Pimentel & Papaj 1996a).

Field Observations

Copulation duration in relation to fruit presence

In the field, pairs in copula frequently depart from the fruit. The goal of these observations was to quantify the frequency of this behaviour and to examine its association with copulation duration under natural conditions. We observed flies on *Juglans major* trees in lower Garden Canyon (1500 m in elevation) in the Huachuca Mountains, Arizona. We conducted observations from 0800 to 1730 hours 3–4 days/ week during 11–27 July 1995. The temperature recorded inside the foliage fluctuated between 16 and 32°C. At temperatures higher than 30°C, the activity of the flies clearly decreased and the observations were suspended.

We recorded copulations by walking around the tree until we detected flies interacting on fruit. We then observed mating activity on the fruit for at least 10 min or until all copulations terminated. For all copulations, we recorded the duration, the density of males and females on the observed fruit and nearby fruit, the time of departure of the pair (if any) from the fruit, and the cause of departure, if apparent.

Laboratory Experiments

The effect of resource presence

In this experiment, we tested the effect of resource presence on copulation duration. We assayed isolated pairs of flies to remove any effect of OSR due to conspecifics other than members of the mated pair. Based on sperm competition theory, we predicted that resource presence should decrease the length of copulation (see Introduction).

We separated the flies by sex and placed them in 48-ml plastic cups containing water, sugar and protein hydrolysate at least 48 h before testing. Each cup contained six or seven individuals that were used only once in every experiment. On the day of testing, we furnished a group of 8–10 48-ml test cups with a 3.7-cm diameter, yellow, plastic sphere suspended with wire from the top of the container. An equal number of cups were left free of spheres. The spheres served as surrogate walnut fruit; on such spheres, males adopt territorial displays and appear to court females as they do on real walnut fruit (which were unavailable when our studies were conducted) (Alonso-Pimentel & Papaj 1996a). Females attempted to lay eggs in the spheres, whereupon they were frequently mounted by males in the same manner as on actual fruit. Moreover, the effects of sex ratio on mating dynamics in assays using surrogate fruit were essentially identical to the effects observed in the field on real fruit (Alonso-Pimentel & Papaj 1996a). Cups were visually

isolated from one another using small, white, cardboard partitions.

We introduced a male into each cup, and within 15 min, we introduced a single female (age 13–23 days) into each cup. We began observations as soon as a test female was introduced into the container. We conducted all observations between 1300 and 1700 hours in a fluorescent-lit room at a temperature of ca. 28°C and at ambient (usually low) humidity. We used a running stopwatch to record the duration of each copulation in each cup over a given test day. We collected data for 23–25 pairs in each treatment.

Interaction between OSR and resource presence

To examine the interaction of a previously reported effect of OSR on mating duration (Alonso-Pimentel & Papaj 1996a) with any similar effect of resource presence, we used a fully crossed design. This design consisted of four treatments: a male-biased environment with surrogate fruit, a male-biased environment without surrogate fruit, a female-biased environment with surrogate fruit, and a female-biased environment without surrogate fruit.

At least 3 days prior to the test, we separated males and females and marked them individually by placing one dot of tempera paint (Proart brand) on the dorsal side of the thorax. Males and females were kept in separate 3.78-litre clear plastic containers with slips of protein hydrolysate, sugar and water. The experimental design was similar to that of the two previous experiments, except that each cup contained a group of flies representing a particular OSR. Male-biased ratios (four males and two females) or female-biased ratios (four females and two males) were alternated systematically. We introduced males into the container at least 15 min before females, and began observations as soon as females were introduced into the container. We conducted all observations between 1400 and 1700 hours in a fluorescent-lit room at a temperature of ca. 28°C and ambient (usually low) humidity. We recorded the duration of each copulation in each cup, noting the identity of each male and female. In this experiment, data were collected for 22–25 trials of each treatment.

Statistical Analysis

In each laboratory experiment, the median value of a variate within a cup was used as a datum in our statistical analysis. Not included in the analysis were the few matings that occurred away from the sphere in cups that contained spheres. Because matings within a cup are arguably not independent of one another, a parallel statistical analysis was run using the first mating of each cup. Analyses of both medians and first matings yielded the same result and we therefore report mainly the median.

In all three laboratory experiments, we compared distributions using the Kolmogorov–Smirnov test. We used a log-linear analysis to test for an association between presence of surrogate fruit, OSR and copulation duration using PROC CATMOD in SAS (SAS Institute 1992). To
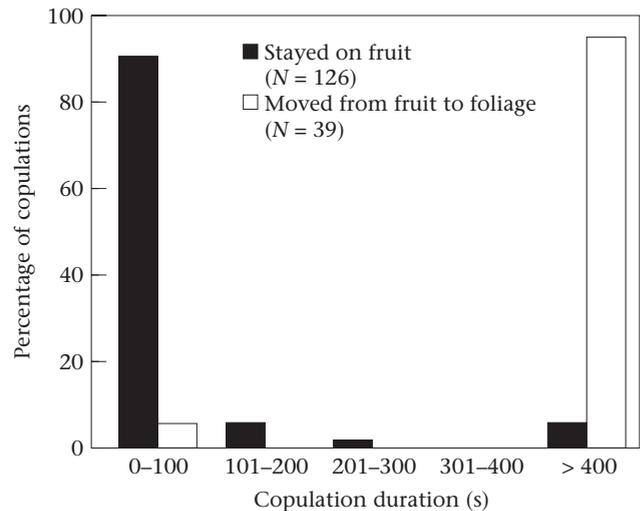

Figure 1. Frequency histogram of field data contrasting the distribution of the copulation duration of mating pairs that stayed on the fruit versus those that moved from fruit to foliage.

determine the best-fitting log-linear model and the significance of the interactions among the variables, we constructed alternative models and compared these using a forward-fitting technique (Knoke & Burke 1980). Different interaction terms were added separately to a baseline model that contained the main effects and the interaction of the explanatory variables (OSR and the presence of surrogate fruit). The significance of each interaction term, in turn, was calculated by subtracting the G^2 values of a model without the interaction term from a model with the interaction term. We followed Zar (1984) and Sokal & Rohlf (1981) for statistical analysis and used SAS (SAS Institute 1992) and SYSTAT (Wilkinson 1992) to run the tests.

RESULTS

Field Observations

Copulation duration in relation to fruit presence

In the field, virtually all observed matings between pairs were initiated on fruit (we only observed one mating from the onset in the foliage). The majority of such fruit-initiated matings were brief (Fig. 1a). However, not all pairs that initiated copulations on fruit completed those copulations on fruit. Of pairs initiating copulation on fruit, 24% ended in the foliage. Leaving the fruit was associated with copulation duration: pairs that left the fruit copulated for significantly longer than pairs that stayed on the fruit (Mann–Whitney U test: $U=4805.5$, $P<0.0001$; Fig. 1b).

Median copulation duration for pairs that moved from fruit to foliage was longer than the median copulation duration of pairs that did not move (median copulation duration of pairs that stayed on fruit=51.0 s, $N=126$; median departure time of pairs that left the fruit=158.0 s, $N=32$, Mann–Whitney U test: $U=3370$, $P<0.0001$).
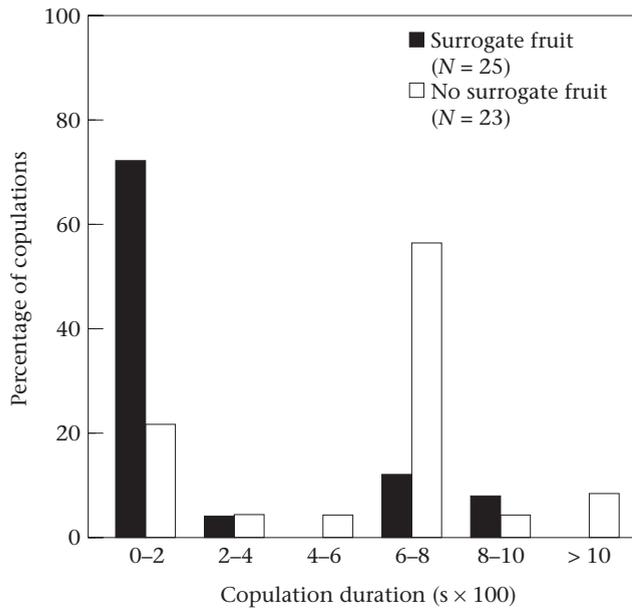
Figure 2. Frequency histogram comparing the distributions of copulation duration for pairs mating in the presence of surrogate fruit versus those mating without surrogate fruit.

For 37 out of 39 mating pairs, departure from the fruit appeared to be due to harassment from other males. Departure appeared, in one instance, to be caused by the arrival of a probable predator (stink bug) and, in the remaining instance, by a strong gust of wind. We used a stepwise multiple logistic regression analysis to assess whether and how male density, female density and the interaction of both gender densities contributed to departure from the fruit. Only male density entered the model as a significant factor in explaining departure (Wald $X^2=10.79$, $P<0.001$).

Laboratory Experiments

The effect of resource presence

In laboratory assays, copulations were more frequent when a surrogate fruit was present, occurring in 50% of the cups with plastic spheres (total number of cups, $N=50$) but in just 23.5% of the cups without spheres (total number of cups, $N=98$). In addition, the presence of a surrogate fruit was associated with a reduction in copulations duration. Exactly 80% of matings in the presence of the surrogate fruit ($N=25$) were short (i.e. <200 s), compared with 17.4% of matings in the absence of surrogate fruit ($N=23$; Fig. 2). The distributions of copulations under each treatment (presence versus absence of surrogate fruit) were statistically different (Fig. 2; Kolmogorov–Smirnov test: $D=0.703$, $P<0.0001$).

The number of copulations was significantly greater (mean ± SE=3.9 ± 0.57) in cups with plastic spheres than in cups without spheres (1.6 ± 0.21; t test: $t=3.73$, $P<0.001$). Thus, treatment differences may reflect a direct effect of territory presence on duration, or an indirect effect mediated through an effect of copulation number.
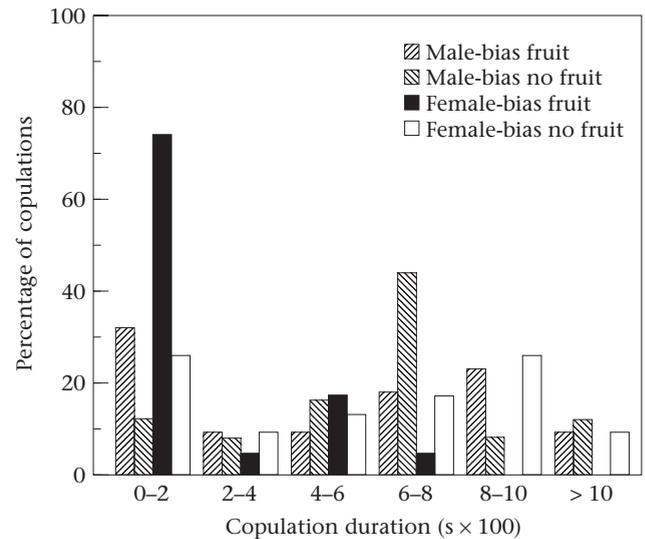

Figure 3. Frequency histogram of the laboratory data showing the four treatments of the fully crossed design of OSR (male bias and female bias) and the presence/absence of surrogate fruit.

Percentage of short copulations

OSR

		Female biased	Male biased
Surrogate fruit	Yes	74% $N = 23$	32% $N = 22$
	No	26% $N = 23$	12% $N = 25$

Figure 4. Matrix of laboratory data showing the percentage of copulation medians that were of short duration (≤200 s), according to the OSR and the presence of surrogate fruit.

Interaction between OSR and resource presence

In the field observations and in the experiments presented above (Figs 1–2), as well as in this experiment (Fig. 3), distributions of copulation duration were predominantly bimodal, having a gap within the range of 200–400 s identical to that found in a previous study (Alonso-Pimentel & Papaj 1996a). For purposes of analysis, we therefore dichotomized copulations as 'short' (<200 s) or 'long' (≥200 s) and present results in these terms.

At a given OSR, the percentage of short copulations increased in the presence of the surrogate fruit (Fig. 4, inspection down columns). This result is in agreement with the result from the single-pairs experiment. Copulations tended to be shorter in female-biased environments than in male-biased environments (Fig. 4; inspection across rows), a result consistent with an earlier study (Alonso-Pimentel & Papaj 1996a).

Results of log-linear models designed to analyse these patterns are presented in Table 1. The difference between

Table 1. Results of log-linear categorical analysis of the association between operational sex ratio, presence of a surrogate fruit and copulation duration in the laboratory

Source of variation	Likelihood ratio		
	G^2	df	P
Model and terms included			
1 L+OSR+BL+(OSR×BL)	22.30	3	0.0001
2 L+OSR+BL+(OSR×BL)+(L×OSR)	13.75	2	0.0010
3 L+OSR+BL+(OSR×BL)+(L×BL)	9.84	2	0.0073
4 L+OSR+BL+(OSR×BL)+(L×OSR)+(L×BL)	0.69	1	0.4070
Difference taken			
Model 1–model 2	OSR 8.55	1	<0.005
Model 1–model 3	BL 12.46	1	<0.001
Model 3–model 4	OSR given BL 9.15	1	<0.005
Model 2–model 4	BL given OSR 13.06	1	<0.001

OSR: Operational sex ratio; BL: surrogate fruit (Ball); L: copulation duration: ≤200 s (short) or >200 s (long).

model 1 and model 2 indicates that the OSR had a significant effect on copulation duration. Similarly the difference between model 1 and model 3 shows that the presence of surrogate fruit had a significant effect on copulation duration. Model 4 (full design) provides an acceptable fit. The good fit of the model shows that both interaction terms simultaneously contributed significantly to the explanation of our data. This result also indicates that the three-way interaction (OSR × surrogate fruit × copulation duration) was not a significant term in our model. In other words, the effects of OSR and surrogate fruit on copulation duration were additive. Note that in the subtraction of the models, the G^2 value for the presence of the fruit was greater than that for the OSR. To quantify this difference, we followed Knoke & Burke's (1980) method in calculating an R^2 analog to determine which model accounted for more of the baseline model variation. According to this analysis, model 2 (OSR effect) accounted for 38.4% of the baseline model variation, while model 3 (surrogate fruit effect) accounted for 55.9% of the baseline model variation.

In this experiment, the mean (± SE) number of copulations was significantly higher in the cups with the surrogate fruit (4.4 ± 0.60) than in the cups without such fruit (2.3 ± 0.24; t test: t=3.25, P=0.002). The number of copulations did not, however, depend on OSR: the mean number of copulations in female-biased cups (3.5 ± 0.46) did not differ significantly from that in male-biased cups (3.1 ± 0.47; t test: t=0.63, P=0.528). We also ran a treatmentwise comparison using first matings and medians of all the matings per cup to test for a possible effect of copulation frequency in our results. The two distributions were not statistically different (Kolmogorov–Smirnov test: D=0.086, P=0.885). Log-linear models using first-matings data instead of medians yielded identical patterns with similar levels of statistical significance.

DISCUSSION

The results of this study corroborate our predictions. Fruit presence and female-biased sex ratios are associated with shorter copulations, as predicted by sperm competition theory. We also found, as predicted, that effects of fruit presence are independent of the effects of operational sex ratio. Below we discuss our results in the context of sperm competition theory.

Effect of Resource Presence

Several studies on insects have reported a tendency for proximity to an essential resource to shorten copulation duration (Parker 1971; Miller 1983; Siva-Jothy 1987; Wolf et al. 1989; Ward & Simmons 1991; Parker 1992). Most authors argue that this pattern, in accordance with sperm competition theory, is a response to the higher encounter rate of territorial males with receptive females at a defended resource (Parker 1970, 1971; Siva-Jothy 1987), and to the correspondingly higher probability that males will gain additional matings (Parker 1970; Michiels 1992). This argument relies mainly on two premises: first, males control copulation and, second, the resource is associated with a higher rate of encounter between the sexes (Parker 1971). The first premise is rarely verified and has not been evaluated in our system. The second premise is supported by casual field observations of *R. juglandis*, but direct quantitative data are lacking. Aside from increasing rates of encounter with females, the resource might also increase the success of male mating attempts. Several studies in *Rhagoletis* (Prokopy & Bush 1972; Prokopy 1976; Smith & Prokopy 1980; Papaj 1994) have suggested that female 'receptivity' is associated with presence at the resource. Females may be more 'receptive' on the fruit because access to the female genital opening and successful copulation by the male requires extension of the ovipositor, as occurs when females are attempting to lay eggs (personal observation). In fact, matings tended to occur as females attempted to oviposit, as has been reported in previous studies (Prokopy & Bush 1972; Prokopy 1976; Smith & Prokopy 1980; Papaj 1994; Alonso-Pimentel & Papaj 1996b).

While resource presence clearly has an effect on copulation duration in laboratory assays, it cannot account for

variability in copulation duration in nature unless matings are at least sometimes initiated away from fruit. Some studies have reported that matings early in the season are initiated in the foliage (Prokopy 1976; Smith & Prokopy 1980, 1981, 1982). Observing such early season matings is difficult. During our observations, only one pair initiating mating in the foliage was recorded from onset of copulation, which was long (795 s). Eight other pairs discovered in the foliage at the beginning of the season had long copulations. These matings presumably were initiated in foliage because females were not yet visiting walnut fruits (Alonso-Pimentel & Papaj 1996b; D. R. Papaj, unpublished data). Early in the season, females generally contain sperm prior to their first appearance on fruit (Alonso-Pimentel & Papaj 1996b). We suspect that matings off fruit occur frequently at the beginning of the fruit season (as reported by Prokopy 1976; Smith & Prokopy 1980, 1981), and are generally long in duration.

Departure from the Resource

Each sex has a distinctive use for the oviposition resource. In general, males defend such resources to obtain matings, while females mostly use the resource to oviposit. Parker (1971) has discussed the male perspective on the adaptive significance of emigration of pairs from the resource. He argued that this strategy reduces the danger that a mating male will be displaced by other males ('take-overs'). Following this rationale, he also predicted that the migration to the surroundings should be directly related to the density of males in the territory.

Support for Parker's arguments was mixed in our study. As Parker predicted, pair emigration from fruit 'territories' was positively correlated with the density of males relative to females. Also consistent with Parker's argument is our observation that harassing males appear to be the most common cause of emigration of the copulating pair from the fruit (male harassment being associated with ca. 95% pairs that emigrated from the fruit; see Results). That this movement is instigated by the male (and thus perhaps functional for the male) is supported by the observation that most copulating *R. juglandis* pairs fly (rather than walk) to the foliage. Parker (1971) notes that, in Diptera at least, flight during copulation is under male control.

Parker's argument that take-overs constitute a major selective pressure on pair movement, could not be confirmed in our study. Take-overs were simply never observed. In fact, although reports of pairs leaving fruit are common in studies of tephritid flies (cf. Smith & Prokopy 1982; Hendrichs & Reyes 1987), take-overs have not been reported to our knowledge in any member of the family. Possibly, copulating pairs always leave the territory before take-overs can occur.

Sperm competition provides a theoretical framework to interpret male interests. Female views, however, are frequently poorly understood. In our system, we lack data on ecological factors such as predation risk, or female foraging success to evaluate female interests on leaving the oviposition resource during mating.

Interaction between OSR and Resource Presence

Studies on interactions among ecological variables affecting mating traits are limited (Sih & Krupa 1995). A major finding in our study is that OSR and territory are strictly additive in their effects on copulation duration, that is, the effect of territory presence did not depend on the effect of OSR. In one of the few studies on interactions of ecological factors, Sih & Krupa (1995), by contrast, found that male density, female density and predation risk interacted in their effects on mating duration in water striders. They explained the complexity of the interactions in terms of male/female conflict of interest in mating. In *R. juglandis*, the male's interests regarding resource presence and OSR are fairly straightforward (Alonso-Pimentel & Papaj 1996a; also see above) in the context of sperm competition. As mentioned above, the *R. juglandis* female's interests are not understood. In this species, it would thus be difficult to determine whether the sexes are in conflict in their mating interests and how any conflict should influence the interaction of resource presence and OSR effects.

The additive effect of resource presence and OSR on copulation duration may reflect a commonality of mechanism. Changes in resource presence or OSR may change the rate of encounter between males and females, a parameter thought to be fundamental to mating decisions from a male perspective (Parker 1970, 1971). If, for example, males adjust copulation duration according to female encounter rate, then the effects of resource presence and OSR on duration will be additive whenever the effects of those variables on female encounter rate are additive.

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