Pitfall Trapping of Male Darkling Beetles Not Induced by Females

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ABSTRACT -- Darkling beetles (Eleodes obsoleta Say, Coleoptera: Tenebrionidae) are among the most abundant and conspicuous macroarthropods of the Great Plains. Most studies of darkling beetle ecology have used pitfall trapping. Speculation suggests that female darkling beetles caught in pitfall traps emit sex pheromones, leading to increased captures of conspecific males and thereby to inflated estimates of population density. However, no published field experiments have quantified this effect. To determine whether the presence of a conspecific female increased the number of males that were captured, live female darkling beetles were placed into twenty pitfall traps. A chi-square test compared the number of conspecific males captured in these pitfall traps over four consecutive days each in both June and July 1997 to the number of males captured in 20 pitfall traps that contained no females. Because no significant differences were found in the number of males captured in female-baited and unbaited pitfall traps, there was no evidence for a female-induced increase in trap attractancy (P = 0.368).

Key words: Eleodes obsoleta, pheromone, attractancy, pitfall trap, Tenebrionidae, Colorado, shortgrass prairie.

Sex pheromones are produced by the females of many insect species. These compounds may directly or inadvertently advertise the location of the female to potential mates. Males may travel great distances towards this chemical beacon to locate females (Willis et al. 1991). Use of such semiochemicals for mate-finding may be particularly important when the spatial location of a conspecific is uncertain, as occurs at low population density or when species are vagile or nomadic (Safonkin 1993, Fauvergue et al. 1998, Logan et al. 1998), as is the case with darkling beetles (Eleodes spp., Coleoptera: Tenebrionidae).

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Several researchers have proposed that female darkling beetles use pheromones to attract mates (Haverfield 1965, Ahearn 1971, Thomas and Sleeper 1977), although direct evidence is wanting and no pheromones or pheromone glands have been identified in *Eleodes*. Other tenebrionid genera produce pheromones (e.g., *Blaps*, Sorour 1995; *Tenebrio*, Paesen and Happ 1995; and *Tribolium*, Obeng-Ofori and Coaker 1990, Rangaswamy and Sasikala 1991). *Eleodes* beetles do produce several chemical compounds (particularly quinones) that deter predators; for more details about the chemistry of these compounds, see Tschinkel (1975). Therefore, it is not unreasonable to suspect that female darkling beetles are histologically capable of producing chemicals that advertise their location for the purpose of attracting mates. This premise has led some researchers to speculate that pheromones thus influence darkling beetle population structure (Haverfield 1965, Ahearn 1971, Thomas and Sleeper 1977).

Darkling beetles are flightless but highly mobile. With ca. 100 species occurring in the deserts, grasslands, and shrublands of western North America (Arnett 1960), they are among the most common macroarthropods of the Great Plains (Crawford 1981). Adult darkling beetles do not possess a true home range and may wander over dozens of meters in a single day (Calkins and Kirk 1973, Doyen and Tschinkel 1974, Whicker 1983, Crist et al. 1992). Adults are generalist herbivores/detritivores (Yount 1971). Adults of both sexes may live one to two years, reproduce throughout that time, and potentially produce two cohorts of offspring. Adults eclose in summer or autumn, reproduce, lay eggs that hatch into soil-inhabiting larvae, overwinter underground, surface the following year during a period that coincides with the emergence of a new cohort of adults, reproduce, lay eggs, and die (Allsopp 1980 and references therein). Female darkling beetles are sexually active throughout adulthood and thus may broadcast pheromones continuously.

Beyond the reproductive consequences of the use of semiochemicals by darkling beetles, there may be ramifications for entomological studies. For example, if a pheromone-emitting female is caught in a pitfall trap, she may cause conspecific males that would otherwise go uncaptured to be attracted and trapped. This female-mediated trap aggregation may potentially bias estimations of population density. Reliable autecological studies of insects must therefore consider the magnitude of this effect and correct density estimates accordingly.

preclude use of the technique to make comparisons across species that may differ in activity (Mitchell 1963, Greenslade 1964, Vlijn et al. 1968, Luff 1975). The presence of bias in autecological studies from female-induced trap aggregation has been presumed (Haverfield 1965, Ahearn 1971, Thomas and Sleeper 1977), but to date there are no published field data that actually assess this effect. Here I report the results of a field experiment conducted in the shortgrass prairie of northern Colorado on Eleodes obsoleta (Say), one of the most widespread and abundant species of darkling beetle, to determine the presence and magnitude of female-induced trap aggregation on estimates of local population size.

**STUDY AREA AND METHODS**

During 18 to 22 June and 9 to 13 July 1997, I conducted my experiments at the National Science Foundation Shortgrass Steppe Site (SGS) in Weld County, Colorado. The SGS as a whole has a diverse arthropod fauna and supports eight sympatric Eleodes species (Bell 1971, Kumar et al. 1976).

I randomly selected a portion of the SGS to establish my experimental plots. This area was approximately 1651 m in elevation and possessed sandy loam soil. Percent vegetation cover at this site was predominantly (96.04%) blue grama shortgrass (Bouteloua gracilis [von Humbolt, Bonpland, and Kunth] Lagasca) (Site 3 in Table 2.1 of McIntyre 1998). Such relative homogeneity in ground cover minimized potential bias in pitfall-trap captures from differences in nearby vegetation (Greenslade 1964, Vlijn et al. 1968).

Eleodes obsoleta was chosen for study for several reasons. It is one of the most abundant darkling beetle species at the SGS (Whicker 1983, Whicker and Tracy 1987, Crist et al. 1992). It is also among the most widespread species within the genus Eleodes, occurring in arid and semi-arid areas from Texas and New Mexico northward to Montana and South Dakota, eastward to Oklahoma, and westward to Arizona (Blaisdell 1909). The sexes are dimorphic, with females being larger than males (average length±SD x width±SD, n = 7: males = 14.0±2.2 x 5.6±0.9 mm, females = 16.3±2.0 x 7.7±1.3 mm) and heavier (average live mass±SD, n = 7: males = 158.9±35.9 mg, females = 179.4±24.6 mg), although note that there is some overlap. Depending on temperature (Whicker 1983, Whicker and Tracy 1987), this species is primarily active from early June to late October. It is largely diurnal at the SGS but becomes more nocturnal with increasing ambient temperature (Whicker and Tracy 1987). Of the eight eleodids found at the SGS, E. obsoleta is the most vagile species, exhibiting no strong microhabitat affinities.
or a home range (Whicker 1983). Thus, this species is an excellent candidate for studies of how pheromones may induce local aggregations of individuals. More information on life history traits of this species and other eleodids may be found in Allsopp (1980), Whicker (1983), and Whicker and Tracy (1987).

Adult female *E. obsoleta* were collected by hand from the SGS ca. 2.5 km from my experimental plots, immediately prior to commencement of my experiments. The reproductive status of eleodids cannot be determined externally (Allsopp 1980). Potential bias from using females of unknown reproductive status was minimized, however, by collecting females at the start of their active season (Whicker 1983, Whicker and Tracy 1987). In addition, a lack of certainty about the mating status of the females I used in my experiments may be of little consequence since I have observed female *E. obsoleta* mate throughout the active season, both in the field and in the laboratory, and individual females will mate repeatedly (unpubl. data).

In my experimental protocol, a live female beetle was placed in a clean, dry pitfall trap (a 500-ml plastic Barber-style trap; see design in Weeks and McIntyre 1997). Twenty such pitfall traps were used (hereafter, "baited" traps). Traps were spaced 3 m apart along four line transects, with five traps per transect (Fig. 1). The replicate transects were separated by 15 m and were placed in alternating parallel and perpendicular orientation so as to minimize potential bias from any prevailing winds that might carry pheromones. Thus, the overall trapping area over which *E. obsoleta* population density was estimated as 828 m² (12 m x 69 m). Because darkling beetles are highly mobile, the effective trapping area may be assumed to be larger than the actual trapping area. How much larger can only be speculated, however, since these beetles do not possess true home ranges (Whicker 1983). Therefore, I used the most conservative area to calculate population density—the actual area without any sort of buffer zone. Traps were checked once daily for four consecutive mornings each in June and July 1997. All captures were removed daily except for the female darkling beetle "bait." If a male *E. obsoleta* was captured, a new female was placed in the trap. Population density of *E. obsoleta* was estimated as the number of individuals captured divided by the trapping area (828 m²). The number of conspecific males captured was noted by date to determine whether there was an increase in trap attractancy over successive days. A four-day trapping period was chosen because it represented an intermediate level of trapping duration, based on prior pitfall-trapping studies of darkling beetles (Rickard and Haverfield 1965, Rickard 1970, Ahearn 1971, Calkins and Kirk 1973, 1975, Rickard et al. 1974, Thomas and Sleeper 1977, Thomas 1979, Whicker 1983, Whicker and Tracy 1987, Parmenter et al. 1989, Crist and Wiens 1995, Krasnov and Shenbrot 1996, 1997).
A chi-square test with Yates' continuity correction for small sample sizes (Sokal and Rohlf 1981) was used to compare captures from the 20 baited traps to those from 20 pitfall traps, located 2.6 km away from the baited traps, that contained no female darkling beetles. These traps were arranged in the same orientation as the baited traps, covering 828 m². Captures from these traps (hereafter, "unbaited") over four consecutive days each in June and July were used to determine the average capture rate of male E. obsoleta in the absence of a female in a trap. Thus, there was a total of 320 trap-nights (20 baited traps x 8 days [4 in June, 4 in July] + 20 unbaited traps x 8 days = 320). The spatial separation of baited and unbaited pitfall traps should not incur artefactual differences from local effects because eleodids are nomadic during their lives and wander over several kilometers. All individuals were subsequently released ca. 3 km away from the experimental area upon termination of the experiment.
RESULTS

No significant differences were found in the number of male *E. obsoleta* captured in traps containing conspecific females from traps containing no females, for either month (June: \( \chi^2 = 0.043, df = 1, P = 0.836 \); July: \( \chi^2 = 1.75, df = 1, P = 0.368 \)). Seventeen male *E. obsoleta* were captured in the baited pitfall traps over 160 trap-nights (5 in June, 12 in July). Captures did not increase in rate with time. In June, three males were captured the first day and the remaining two were captured the third day. Each of these males was caught in a different trap. In July, 4, 4, 1, and 3 males were captured over the four days, respectively. Two of these males were caught in the same trap (first day). Nineteen male *E. obsoleta* were captured in the unbaited traps (5 in June, 14 in July). Daily captures over the four days were 1, 2, 1, and 1 males in June and 4, 3, 3, and 4 males in July.

The population density of *E. obsoleta* in the baited pitfall trap area was estimated as 0.006 individuals/m\(^2\) in June and 0.014 individuals/m\(^2\) in July; the population density of *E. obsoleta* in the unbaited pitfall trap area was estimated as 0.006 individuals/m\(^2\) in June and 0.017 in July. These estimates are slightly lower than density estimates obtained by Whicker (1983) and Ebert (1990) for *E. obsoleta* and several congeneric species in the same location of the SGS where my experiment was conducted. Their population size estimates ranged from 0.040 to 4.70 individuals/m\(^2\).

Some other darkling beetle species were also captured in both the baited and unbaited traps. In June, one male *Eleodes fusiformis* LeConte and one female *Eleodes hispilabris* Say were captured in two baited traps on the fourth and third days, respectively, while one male *E. fusiformis* was captured in an unbaited pitfall trap on the first day, and one female *E. fusiformis* and one male *Eleodes extricata* Say were captured in unbaited traps on the second day. In July, the baited traps yielded a female *E. obsoleta* (first day) and two *Eleodes obscura* Say (sexes unknown; second and third days); unbaited traps contained one *E. obscura* (sex unknown; fourth day), one female *E. fusiformis* (second day), and two *E. extricata* (one male, one female; second day).

DISCUSSION

The presence of a female *E. obsoleta* darkling beetle in a pitfall trap did not make the trap more attractive to conspecific males, even after four days, in either month. Thus, population density estimates were not inflated by the presence of females in pitfall traps.

The lack of evidence for female-induced pitfall trap attractancy does not mean that female *E. obsoleta* do not produce or use pheromones to attract males, however. Many insects release pheromones only under certain
circumstances, primarily when females are unmated. If female-induced pitfall trap attractancy is strongest early in the active season when most of the virgin females emerge, estimates of darkling beetle population size would be most biased when I conducted my experiment, but no such bias was detected. Despite the uncertain reproductive status of the females I used, my experimental design represented a scenario that is potentially faced by every entomologist who uses live pitfall trapping, since most estimates of species diversity and population density obtained from pitfall trapping disregard the reproductive status of captured individuals. Many insects produce pheromones only (or more intensely) under certain environmental conditions, e.g., at specific temperatures. Although pitfall-trapping studies of darkling beetles may not recreate these conditions consistently, it is important to assess any potential pheromonal bias such studies may experience.

Speculation that darkling beetles use pheromones may have been fueled by observations of mating by captured conspecifics. Indeed, I observed one pair of *E. obsoleta* mating within a pitfall trap (pers. observ.). Such matings may simply be a passive consequence of pitfall trapping in general, with males mating with females with whom they come in contact regardless of how that contact came about, and not a consequence of females actively luring males. The relatively low numbers of congeneric species captured in the baited pitfall traps indicate that if female *E. obsoleta* do produce pheromones, they are no more attractive to conspecific than to congeneric males. The apparent lack of a strong pheromone response in *E. obsoleta* implies that this species should not exhibit local aggregation, as is seen in species that produce and respond to sex pheromones, e.g., several moth species (Bierl et al. 1970, Schneider 1975). Instead, individuals should be rather widely and evenly dispersed. This dispersed pattern of distribution has indeed been observed for this species (Whicker 1983). No chemical has been isolated that has been proven to act as a pheromone in darkling beetles. These facts, coupled with the lack of significant aggregation to traps in my study, suggest that estimates of population size of darkling beetles from pitfall trapping likely suffer little female-inflated bias.

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