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INTERMALE VARIATION IN AGGREGATION PHEROMONE RELEASE IN *Prostephanus truncatus*

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Abstract—Intermale variation in pheromone signaling has been confirmed and quantified by measurements of pheromone produced by single adult male *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). Males varied in both the amounts of the two components of their aggregation pheromone and the ratio of one component to the other. The mean rates of production of the pheromone components T1 and T2 were 1.9 and 0.5 μg/day, respectively. There were repeatable differences among males in the amounts of T2 produced and the proportion of T1 in the pheromone blend over two weeks. Of the 15 males studied, one released a large burst of pheromone in a short period, while the remainder, if they did release, did so over an extended period. This suggested that there may be two alternative release strategies and the significance of this is discussed.

Key Words—Aggregation pheromone, individual variation, sexual selection, larger grain borer, *Prostephanus truncatus*.

INTRODUCTION

The larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), is an important pest of farm-stored maize and dried cassava in Africa and Central America (Hodges, 1986; Markham et al., 1991). Once a male has reached a suitable food source, an aggregation pheromone is released consisting of two components, 1-methylethyl (*E*)-2-methyl-2-pentenoate and 1-methylethyl (*E*,*E*)-2,4-dimethyl-2,4-heptadienoate (Cork et al., 1991; Dendy et al., 1991), given the trivial names Trunc-call 1 and Trunc-call 2 (T1 and T2), respectively. Natural and synthetic pheromone are attractive to both females and males in the laboratory

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(Boughton and Fadamiro, 1996; Hodges and Dobson, 1998) and field (Leos-Martinez et al., 1995; Scholz et al., 1997; Hodges et al., 1998). Synthetic pheromone is used as a lure in traps to monitor the pest (Richter and Biliwa, 1991; Fandohan et al., 1992; Pike et al., 1992) and also *Teretrius nigrescens* (Lewis) (Coleoptera: Histeridae), a predator of *P. truncatus* (Rees et al., 1990; Boeye et al., 1992).

It has been proposed that male *P. truncatus* produce aggregation pheromone primarily to attract females as potential mates and that other males respond opportunistically by aggregating, representing a fitness cost to the signaler in terms of increased competition for mates and possibly for food (Hodges et al., 1999). This hypothesis is supported by the observations that aggregation pheromone is only produced by males (Cork et al., 1991), males shut down signaling with pheromone after adult females arrive (Smith et al., 1996), and the sex ratio of beetles caught from the field is female-biased (Scholz et al., 1997; Hodges et al., 1998). Both laboratory and field bioassays have shown that *P. truncatus* can distinguish between the pheromone signals of different males and that some males are more attractive than others (Birkinshaw and Smith, 2000). The signals of individual males have never been chemically quantified, however. The temporal stability of signals is also unknown. Birkinshaw (1998) suggested that the relative attractiveness of a range of males was consistent over three days, but after five days, this order had changed (although insects were moved between jars of medium in between time).

In this study, we measured the signals from 3- to 5-day-old adults for 24 days in order to determine the degree of intermale variation in signaling and the stability of intermale differences over time, given fairly constant conditions.

METHODS AND MATERIALS

The beetles used were adults of a Ghanaian strain collected from the field in 1996 and cultured on yellow maize in a CTH room at $27 \pm 1^{\circ}C$, $60 \pm 5\%$ relative humidity on a 12-hr light–dark cycle. Newly emerged virgin adults, 1–2 days old, were removed from pupal cells. Males were selected according to the form of the clypeal tubercles (Shires and McCarthy, 1976) and placed on wheat flour for two days. Just before the start of pheromone collection, each male was placed on a single maize grain since males only produce pheromone in the presence of food (Cork et al., 1991; Smith et al., 1996). To provide the beetles with easy access to the maize, each grain was drilled to give a single, blind-ending tunnel (2 mm diam.).

Pheromone was collected from individual males under the same conditions of temperature and relative humidity. Beetles in maize grains or control grains without beetles were placed singly in glass vessels of 30 cm^3 capacity (Fisher Scientific) through which air was drawn at a rate of l000 cm^3/min by electrical diaphragm pumps (Capex Mk II; Charles Austin). The intake air was purified by passage

through a filter containing activated charcoal (20 × 2 cm, 6–18 mesh), and the output air was passed through filters containing Porapak Q (200 mg, 50–80 mesh; Phase Separations) to collect volatiles emitted. A glass round-bottomed flask (1000 cm^3) was also connected in the system, between the pump and collection filters, to act as a buffer against pressure variations induced by the pump. The air was pumped continuously during the test and the Porapak filters were changed at intervals of one, two, or three days over a period of 24 days (see Figure 1 below). Studies with two Porapak filters connected in series showed that both T1 and T2 were fully retained on the first filter for at least three days. Breakthrough of T1 was apparent after six days, but there was no breakthrough of T2 after 13 days.

Volatiles collected on the Porapak were eluted with dichloromethane (750 μl; Fisher Scientific Distol grade) and octyl acetate (5 μg) added as an internal standard. Samples were assayed by gas chromatography, using a fused silica capillary column (30 m × 0.32 mm ID) coated with CPWax 52CB (Carbowax equivalent; Chrompack), with helium carrier gas and flame ionization detection. The temperature was held at 60°C for 2 min, then programmed at 6°C/min to 230°C. The injector temperature was 200°C, and the detector temperature was 240°C. The sample (2 μl) was injected splitless and not concentrated to avoid loss of pheromone components. Data were captured and processed with EZCHrom V.6 software. Amounts of the two pheromone components were calculated by comparison of peak areas with that of the internal standard and application of the corresponding factors. The latter were derived by calibration of pure synthetic components against the internal standard at the 5-μl level. Peak identity was confirmed at intervals by gas chromatography–mass spectrometry (GC-MS; Finnigan MAT ITD700, Thermoquest) using similar chromatographic conditions. The threshold of minimum detectable quantities of pheromone was determined by visual estimation of the magnitude of the smallest reliably detected peaks.

In the first test, there were nine beetles and one control, and this test was then repeated. The results of the two tests were similar (i.e., did not form two distinct data sets, see Figures 2 and 3 below), and for statistical analysis they were combined, giving a total of 15 observations on male pheromone release, since three sets of data were missing due to mortality and a failure in sexing, and the two controls. The repeatability (r) of male differences in signal characteristics, both the absolute amounts of T1 and T2 and proportion of T1 in the blend, was calculated as r = variance between individuals/(variance between individuals + variance within individuals) (Du et al., 1987). The significance of the temporal stability of differences between the same male signal characteristics, absolute amounts of T1 and T2, and proportion of T1 in the blend were assessed by calculating the correlation coefficient between signals produced on day 7 and those produced by the same males on day 21. These days were chosen as they encompassed a period of steady pheromone output for most males.

RESULTS

During the first three days that the beetles were on maize, the pheromone collected on filters was below reliably quantifiable amounts and was estimated to be at or below 10 ng/beetle/day of either component (Figure 1). From day 5 onwards, detectable amounts of pheromone were released by most beetles (Figures 2 and 3) over extended periods, but in one case there was a very large burst of release. In this case, some 70 μg of T1 and 22 μg of T2 were released over a period of only two days, followed by little or no further production. The total amount released by this beetle during the period of study was greater than any other male (Figure 2 and 3), although similar to the 24-day cumulative values of several other males that released 50–60 μg of T1 and 15–20 μg of T2. No detectable amounts of pheromone were observed in the controls.

On average, almost four times as much T1 was produced as T2 (Table 1). The relative proportions of T1 and T2 were less variable than the absolute amounts of T1 and T2 among males. Furthermore, the differences that did exist in this blend among males were more repeatable than the differences in amounts of T1 or T2 (Table 1; Figure 4).

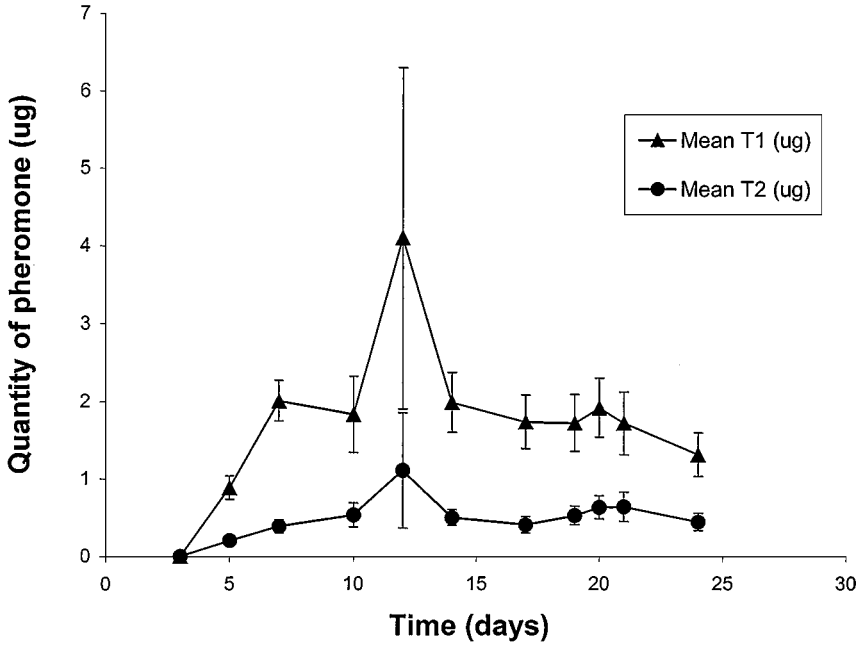

FIG. 1. Mean rate of pheromone output from male *P. truncatus* for pheromone components T1 and T2 (μg/day). Error bars are standard errors of the mean $N = 15$.

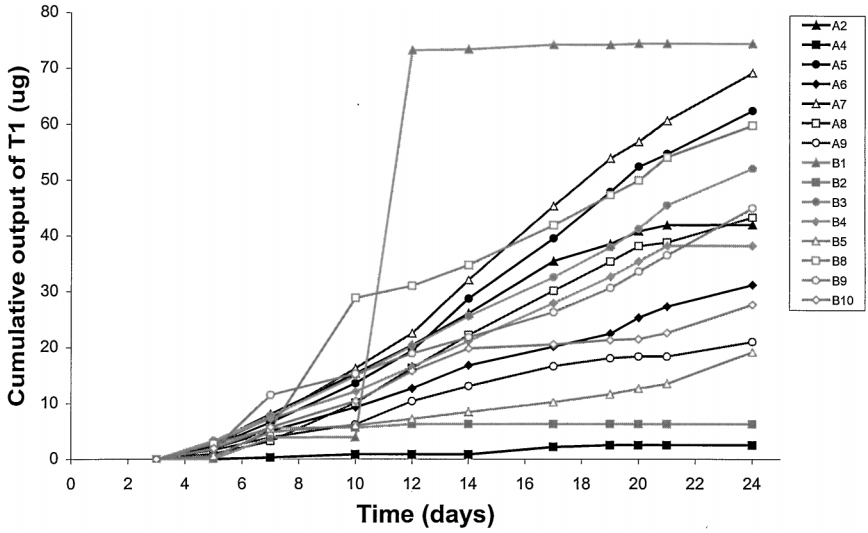

FIG. 2. Cumulative output of pheromone component T1 plotted individually for each male *P. truncatus* (A = first test, B = second test).

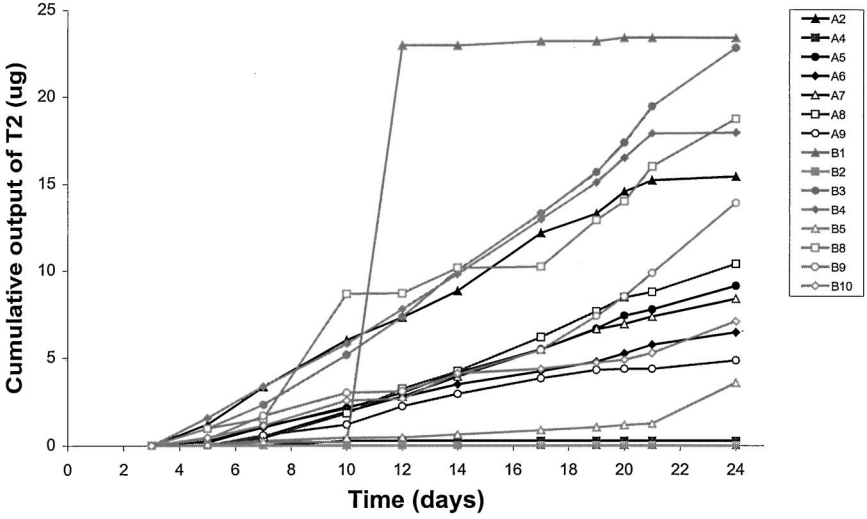

FIG. 3. Cumulative output of pheromone component T2 plotted individually for each male *P. truncatus* (A = first test, B = second test).

TABLE 1. SUMMARY STATISTICS OF QUANTITIES OF PHEROMONE COMPONENTS T1, T2, AND PERCENTAGE OF T1 IN AGGREGATION PHEROMONE BLEND PRODUCED BY INDIVIDUAL MALE *P. truncatus*

	T1	T2	T1[a]
Mean (of each male mean)[b]	1.9 (μg/day)	0.5 (μg/day)	81%
Coef. variation among males[b]	58%	75%	12%
Repeatability among males (r)[c]	0.44	0.49	0.57
Correlation coefficient between day 7 and day 21	0.32 ($N = 15$, NS)	0.57 ($N = 15$, $P < 0.01$)	0.69 ($N = 11$, $P < 0.005$)

[a] Only data where males produced a detectable signal were included.
[b] Using all data from day 5 onwards.
[c] Using data from day 7 to day 21.

No significant correlation ($r = 0.28$, $df = 14$, $P > 0.1$) was found between the rate of total pheromone production and the ratio of the two components among males (Figure 5).

DISCUSSION

During the first three days that male *P. truncatus* were placed on food, rates of pheromone production were below reliably quantifiable amounts in this study

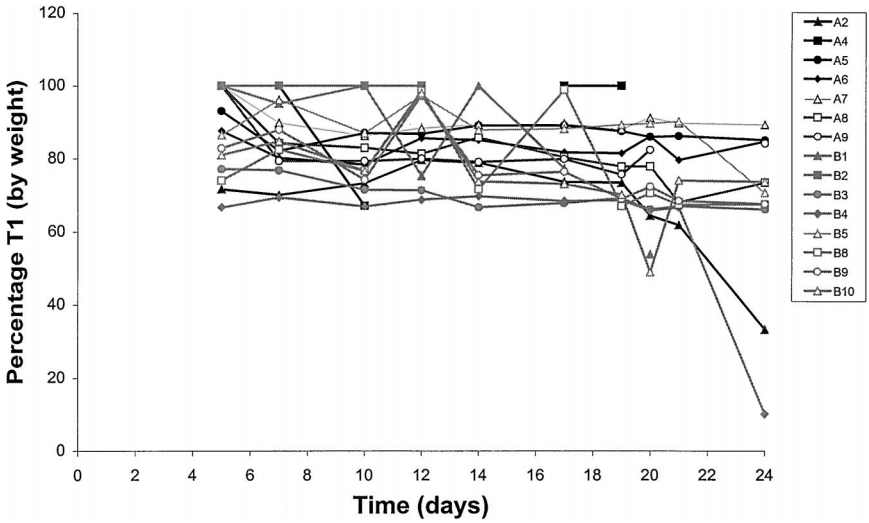

FIG. 4. Percentage of pheromone component T1 in the blend (by weight) plotted individually for each male *P. truncatus* against time.

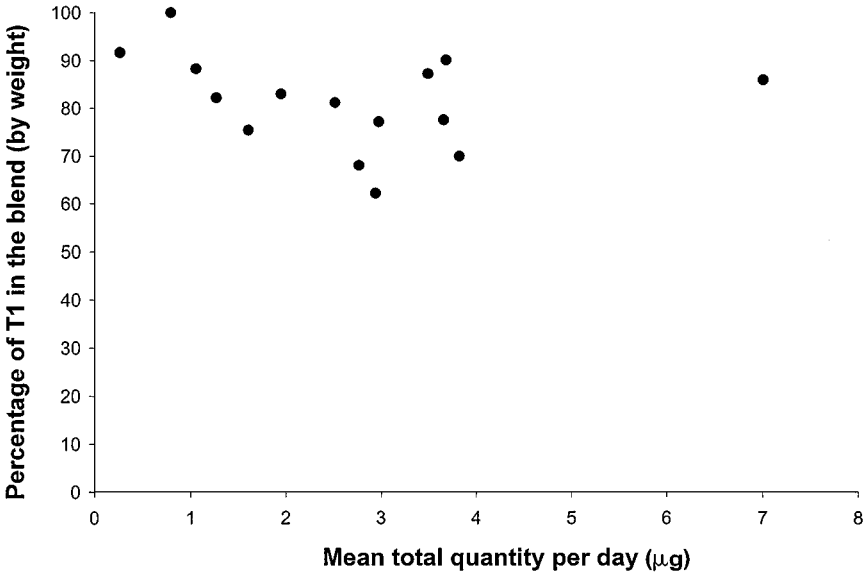

FIG. 5. Mean percentage pheromone component T1 in the blend vs. mean total quantity (T1 + T2) produced per day for each male *P. truncatus* for those days where measurable quantities of pheromone were produced.

($\leq$10 ng/beetle/day). However, bioassay studies have shown that both female and male *P. truncatus* respond to males that have only been present in grain for 24 hr (Hodges and Dobson, 1998); thus, it seems that the beetles respond to quantities of pheromone below our detection limits.

For most beetles, pheromone release extended over the 24 days of the study; however, one male released a single large burst of pheromone in this time. It is possible that this represents an alternative signaling strategy. The average weight of pheromone (T1 + T2) produce by males was 2.4 μg/day, and as the average weight of a beetle is about 3.5 mg (S. Addo, unpublished data), this amounts to 0.06% of body weight. However, in the extreme case, 92 μg was released in a two-day burst. This amounts to a daily rate equivalent to 1.3% of body weight. Little pheromone was produced after the large burst, suggesting that the high energy-costs involved could not be sustained, although this male did not release much more pheromone in two days than several others did in 24 days. If the pheromone burst is indeed an alternative strategy, then presumably the short attraction time is compensated for by the attraction of mates from greater distances. There may be more subtle advantages in avoidance of predation by the histerid beetle *T. nigrescens*, which shows a kairomonal response to *P. truncatus* pheromone (Rees et al., 1990; Boeye et al., 1992). However, this may only be clarified once host location by the predator is better understood.

Intermale variation in aggregation pheromone signaling, as suggested by the behavioral studies of Birkinshaw and Smith (2000), has now been confirmed directly, strengthening their hypothesis that features of the aggregation pheromone signal could be sexually selected. Compared to acoustic or visual signals, direct quantification of individual pheromone outputs are rare (but see Pope et al., 1984; Pavis and Barre, 1993; Miklas et al., 2000; Zhang et al., 2000). Recently, however, the pheromone release from a relative of *P. truncatus*, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), has been measured (Bashir et al., 2002). The male-produced aggregation pheromone of *R. dominica* also has two components, dominicalure 1 (D1) and dominicalure 2 (D2), and chemical structures are similar to those of T1 and T2 (Williams et al., 1981). Indeed, *P. truncatus* is attracted to traps baited with D1 and D2 (Hodges et al., 1983). Comparing the data sets, *P. truncatus* and *R. dominica* produce pheromone at comparable rates (typically 2–4 μg/day). The percentage of component 1 (D1 or T1) in the blend is lower from *R. dominica* than from *P. truncatus* if the components are compared by weight or in terms of the number of molecules of each. The absolute amounts of T1 and T2 are more variable than those of D1 and D2 reported by Bashir et al. (2002), although their ratios have about the same stability. The more variable values for *P. truncatus* were not attributable simply to the large burst of pheromone release in one of the males. Variation in pheromone outputs among male *R. dominica* has been shown to be connected to differences in the body weight (Bashir et al., 2002). Unfortunately, in the current study, the weight of the male *P. truncatus* was not recorded, although in previous work no link could be demonstrated between the weight of males and the attractiveness of their volatile pheromone signals (Birkinshaw, 1998).

The aggregation pheromone signals of male *R. dominica* are phenotypically plastic and can be altered by placing males on different plant hosts (peanuts compared to maize) and by crowding a male with many females (Bashir, 2000). It is already known that *P. truncatus* males greatly reduce their signal in the presence of females (Smith et al.,1996). The possible influences of plant host type on pheromone release by *P. truncatus* are as yet unknown, although males signaling on cassava were found to be less attractive than those signaling on maize in a walking bioassay (Birkinshaw, 1998).

The next logical step is to link chemical quantification of individual pheromone blends to bioassay data to find out which blends are the most attractive. This was recently attempted in *Rhyzopertha dominica* (Bashir, 2000). However, features of the blends, e.g., the ratio between the components, were not good determinants of responders' choices. However, in the case of *P. truncatus*, there is evidence that the blend is important. Artificial lures holding a total of 2 mg of T1 and T2, at a ratio of 2:1, released a blend that is a closer mimic of the natural pheromone than at a ratio of 1:1. Flight traps loaded with the 2:1 ratio lures captured significantly more beetles (Hodges et al., unpublished data).

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