

THE FUNCTIONAL SIGNIFICANCE OF AGGREGATION PHEROMONE
IN *PROSTEPHANUS TRUNCATUS*
1st Year Report (Jan 1996)

Lucy Birkinshaw
Supervisor: Prof. R. H. Smith

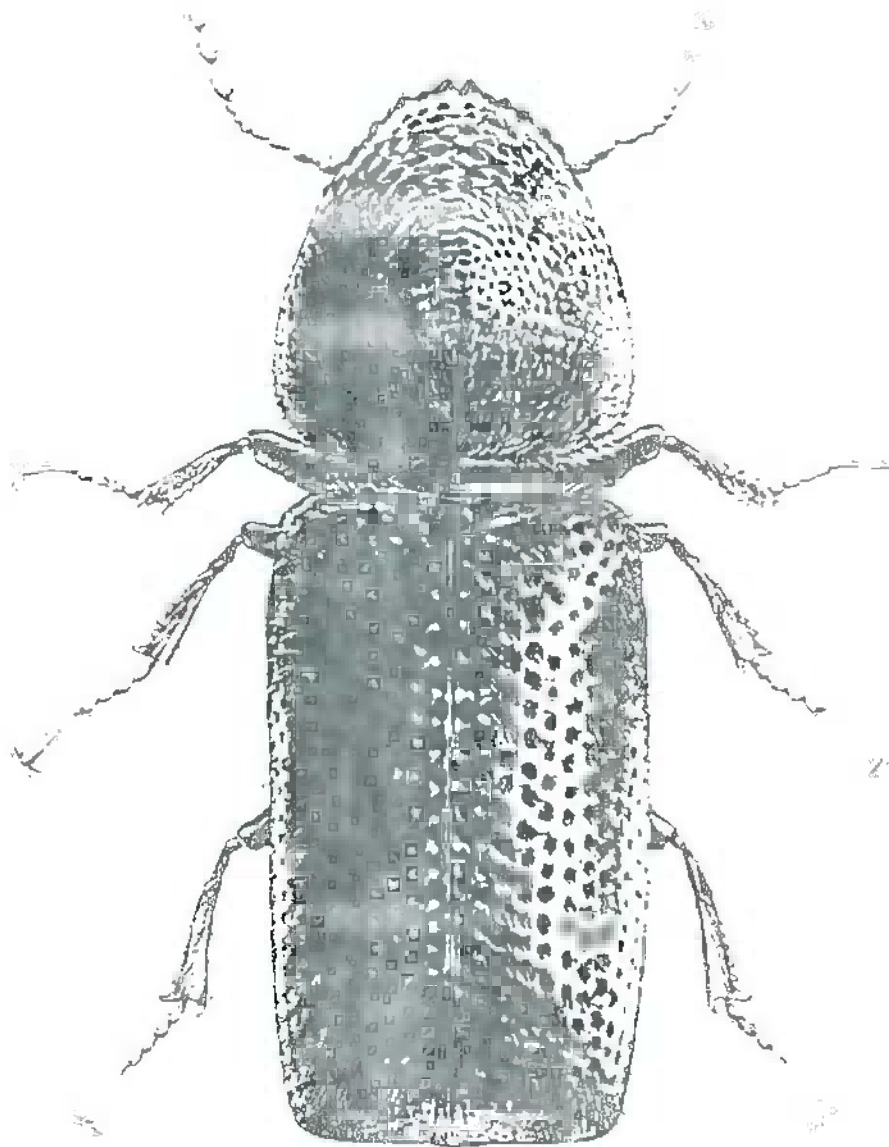


Fig. 1.

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GENERAL INTRODUCTION

***Prostephanus truncatus* (Coleoptera: Bosfrichidae)**

Prostephanus truncatus is a wood and grain borer (Often referred to as Larger grain borer) which lives in tropical climates (see Fig. 1). Adults will make test tunnels in many substrates and females will oviposit at the ends of a tunnel network once the medium is deemed suitable. In maize, eggs are laid in batches of 3-14 and mostly develop within the grain (Li 1988). Adults emerge approximately 4-5 weeks later and after a few days become fully active and ready to mate. This species has a relatively long fertile period of about four months on maize where females can consistently lay about 3-9 eggs per day pausing only to gain entry into new hosts, disperse and/or locate mates. A female requires at least 2 or three separate matings to fertilise all her eggs (Li 1988), but will copulate many more times than this under experimental conditions (personal obs.).

Potential hosts are known to include the dead, drying out underside of branches of certain tree species, maize grain and cassava (Hodges 1986 and 1994). It is the beetle's capacity to breed on maize in particular which has led it to be classified as a pest. Indigenous to Central America and Mexico, *P. truncatus* has recently spread throughout Africa since first detection there in the early eighties (Dunstan and Magazini 1981 quoted in Fadamiro 1995). Damage to stored maize can be particularly severe. In Tanzania, Hodges (1983) recorded a loss of 34% of dry weight after 3-6 months in storage. The distribution of *Prostephanus truncatus* is particularly clumped, with neighbouring stores exhibiting dramatic variation in the level of infestation. This is attributed to the action of a pheromone system (see Hodges 1986).

Male *Prostephanus truncatus* are known to secrete a pheromone consisting of 2 components (Truncall 1 and 2) when feeding on maize. This pheromone attracts both male and female adults and can therefore be classified as an aggregation pheromone. Both truncall 1 and 2 have been chemically identified. Traps containing one or a blend of both components have been trialed for their efficiency in catching both flying and walking insects (see review by Hodges 1996 unpubl.). Results are inconclusive as to whether one component is more attractive than the other or a blend of both. Such comparisons are hampered by the different physical properties and natural emission rates of the components. Truncall 1 (T1) is more volatile and is produced in greater quantities by males than truncall 2 (T2). It is possible that the two components differ in their range of action with T1 acting primarily at short range and T2 acting at longer ranges. T2 does appear to show higher catches in flight traps (Leos-Martinez 1995), T1 doing better in crevice traps (Trapping walking beetles)(Hodges 1996 unpubl.). Misuse of traps could potentially draw insects into grain stores from the surrounding area, acting to infect stores instead of passively monitor for the presence of beetles. Current advice is to locate pheromone traps well away from stores and to use other methods to monitor the within store populations (Hodges 1996 unpubl.). However, if the short/long range hypothesis is correct, then T1 might be a suitable short range lure which could safely be used for within store monitoring.

As discussed in the enclosed review (aim 1), the benefit, if indeed there is one, of the signaller to signal is as yet unknown. Hodges (1994) proposed a male attraction function which has subsequently been criticised by Fadamiro (1995). Fadamiro argues against a primarily sexual function of pheromone since patterns of response appear to be independent of mating experience. The presence of females does, however, seem to affect pheromone production (Smith 1995). Fadamiro proposes that the presence of females may be used by the male as a general indicator of population density. Therefore, males may be altering their behaviour in response to population density and not specifically the availability of a mate.

This study starts with a hypothesis in accordance with Hodges (1994) that pheromone is produced by males primarily as a sex attractant. Initially, male signallers may benefit from attracted males through increase in the efficiency of female attraction. However, males attracted will also represent a cost in terms of increased competition for both mates and food resources. I tentatively extend this hypothesis and propose that the pheromone signal itself is a sexually selected character which may contribute to a pattern of assortative mating.

Sexual selection

Discussed by Darwin (1859), sexual selection is a form of natural selection where the selecting factors are either competition for mating opportunities between members of the same sex (intrasexual selection) or the conflict in interests in mate choice between the sexes whereby the salesmanship of one sex is pitted against the sales resistance of the other (intersexual selection). Sexual selection is apparently identified as separate from natural selection because it can give rise to features which can be maladaptive in other contexts. However, selective pressures are often geared in different directions. Maybe it is the relatively influential nature of sexual selection which has given rise to it's apparently special treatment, acting as it does, so directly on the long term reproductive success of individuals (see Otte 1979 in Blum 1979 for a historical review of sexual selection theory).

A key influence governing patterns of sexual selection is the relative investment of resources from each sex into the offspring (Trivers 1972). Where one sex invests more into parental effort than the other, a conflict of interests is immediately set up whereby the sex investing less has the potential to pass their gene set onto many more offspring than the average. Generally, females allocate relatively more resources to production and care of offspring and males invest more into mating effort. Therefore intrasexual selection is likely to be more important for males than for females. Females are likely to be egg limited and are likely to benefit by fertilising their eggs with sperm of superior genetic quality. A female may also gain non genetic resources from matings, like nutrients in the ejaculate and parental care. The degree to which individuals can actually dictate who they mate with and which sperm is used in fertilisation is obviously limited by constraints such as availability of information regarding mate quality, access to mates and ability to resist/force other individual's behaviour.

In *P. nuncatus* any pattern of assortative allocation of paternity (consequence of sexual selection) could be determined from properties of the male pheromone (potentially fairly long range), properties of the behaviour/physical characteristics of the male (contact necessary), or lastly by properties of his ejaculate (post copulation) commonly referred to as sperm competition.

Sperm competition

Insects are particularly likely to show sperm competition as certain aspects of their biology lend themselves to it (see Parker 1970 for a general discussion).

STORAGE OF SPERM: Many female insects store sperm in a storage organ called the spermatheca.

LONGEVITY OF SPERM: Sperm is often relatively long lived in insects, in many cases it derives nutrients from the female and persists in a viable state for some months. (See Ridley 1988).

MULTIPLE MATING: Insects are often generally promiscuous and as such sperm from one male is less likely to monopolise a female.

Copulation, fertilisation and oviposition are often temporarily separated events in insects and the relative order of mating could influence paternity. P_2 is the proportion of offspring fathered by the second male by a female who has mated with two different males and is commonly used as a measure of relative paternity in such situations. Gwynne reviewed sperm competition patterns in 37 insects (in Smith R L (ed.) 1984)

and found that 3 showed no precedence between the 2 males, 7 showed first male precedence ($P_2 < 0.5$), but 27 showed last male precedence ($P_2 > 0.5$). Zeh (1994) have found that when females are mated with more than two males such order effects are scrambled. They propose that, "...post copulatory sexual selection may be much greater in nature than is evident from two-male mating experiments.". Predictions of paternity allocation depend on the mechanism of sperm competition (Parker 1990). Potential mechanisms of sperm competition are reviewed in Birkhead (1990).

AIMS

1. Review existing literature on Coleopteran aggregation pheromones.

2. Evaluate the potential for sexual selection during direct contact between individuals (2a) and within the female reproductive tract (2b). Very little has been published on the mating system in *Prostephanus truncatus*. This must be studied before the role, if any, of aggregation pheromone in the sexual behaviour of this insect can be evaluated.

3. Describe the pattern of pheromone production as influenced by variables such as time of day physical condition of the signaller and the influence of conspecifics.

Basic patterns of production and response to pheromone must be determined and discussed in terms of what is already known about the insect. This may provide some circumstantial evidence as to the possible functions of this pheromone. Also, basic knowledge is required for practical reasons to aid in the design of later experiments.

4. Determine if there is individual variation in attractivity via pheromone emission between males.

Detection of variation, be it environmentally determined or, more particularly, genetic in nature, is a crucial goal in any evolutionary based study such as this. Variation may be expected to be low for characters with high fitness consequences (ref. Fishers paradox), and this may be the case for pheromone production. However, variation among sexually selected characters may be relatively high. So for the system I am considering, we might expect pheromone to be produced by the vast majority of males (highly selected for), yet blend or some other more subtle property may have larger variation which may be discernible to conspecifics. On the other hand, it is also possible that pheromone production is highly variable and negatively correlated to response to other male's pheromone. In other words males may be primarily signallers OR responders, variation possibly existing both between individuals and also at different times for the same individual. The idea that males of low quality might adopt different reproductive strategies is not new. A male who acts primarily a responder would be performing a "satellite strategy" as described in Hughes (1982).

5. Formulate and test more detailed predictions arising from the results of the aims above.

I will now present the key questions which need to be answered to achieve my aims. Some background or explanations are given where necessary. I will describe some of the methods I have already used and also those I intend to follow in the future to answer them. In the interests of brevity, some methods are omitted and only a very basic description of most methods are given. The results achieved so far follow with some discussion. The same number system is retained throughout to allow easy cross referencing.

KEY QUESTIONS AND METHODS

1. See appendix 2. "Coleopteran aggregation pheromones".

2a.1. How readily will females copulate with many different males?

2a.2. How readily will males copulate with many females?

2a.3. Will females mate more readily with some males than others?

Females will often run at males pushing them to the extent that some are even rolled over several times. This seemingly aggressive behaviour may be used as some measure of female choice between males.

2a.4. Do males demonstrate aggressive behaviour towards other males?

2a.5. Is there any sign of mate guarding behaviour in males?

2b.1. Do female *P. truncatus* possess sperm storage organs?

2b.2. What is the P_2 value for *P. truncatus* and how does it vary between individuals?

A sterilisation method as employed by Eady (1991), will be employed to begin to answer this question. Using this method females are mated with two males, one previously sterilised and one fertile. By counting the numbers of sterile to fertile eggs an estimation of P_2 (proportion of paternity secured by the second male to mate) can be made. There are many limitations to this method, (see Zeh 1994 and Eady 1991) but it is hoped that it will provide some information, particularly of the potential cost to signalling males incurred from the attraction of male competitors.

2b.3. Do males alter their investment in ejaculate in response to perceived levels of inter-male competition in the form of pheromone?

Males have been shown to alter their reproductive investment (in the form of size of ejaculate) in response to perceived levels of inter male competition (for instance via direct contact with varying numbers of males). This has been shown to be the case in both the house cricket, *Acheta domestica* and the decorated field cricket, *Gryllodes supplicans* (A Gage 1996 in press). Similar results have been obtained in humans (Baker and Bellis (1989), rats (Bellis et al 1990), the Mediterranean fruit fly, *Ceratitis capitata* (Gage 1991) and *Tenebrio molitor* (Gage 1992) (All cited in Gage 1996 in press).

3. In order to study patterns of pheromone production and response, a new bioassay is being developed which can detect the response of single responders to single male sources (see Fig. 2 in appendix 1). This can later be adapted to incorporate artificial pheromone sources to control for variation in pheromone production, and direct collection of volatiles to control for variation in response, to separate these two influences on the results obtained. No bioassay for *Prostephanus truncatus* has ever been used to detect a response to pheromone produced by a single male. Ultimately, it is hoped that this will allow any intraspecific variation in characteristics of pheromone production and response to be described. I intend to answer questions 3.1 through to 3.8. using this bioassay.

3.1. How does pheromone production and response vary with the light regime used in the laboratory?

Beetles are cultured on a 12 hour light /dark regime which could potentially create a periodicity of pheromone production. Beetles have been shown to have a periodicity of flight activity under such conditions (Fadamiro 1995).

3.2 How does pheromone production and response vary with the age of insects?

The bioassay could easily be used to describe in relative detail the pattern of onset of pheromone production as beetles emerge, and the effect of age on attractivity for individual males could be investigated. Likewise, the response of females with age could be determined.

3.3. How does the response to pheromone vary with the nutritional state of responders?

3.4. Is tunnelling a pre-requisite for pheromone signalling?

Since food surrounding the signaller may act to smooth any pattern of pheromone emission (see 3.1 results), I have investigated whether males will signal on ground maize (where they would be unable to form discrete tunnels) from which they could be

removed with less disturbance. In other words, does the physical nature of food presented affect pheromone production?

3.5. How is pheromone production affected by placing the signaller on fresh grain?

3.6. How does long term exposure to pheromone influence response and production?

Response:- Obengofori (1990) have already shown that responders can lose sensitivity to pheromone after prolonged exposure.

Production:-The effect of exposure of the pheromone produced by other males on a signalling male could also be investigated as this may influence production (see aim 1 anti-aggregation pheromone section).

3.7. How does response to pheromone vary with the mating experience of insects?

Obengofori (1990) and Fadamiro (1995) have found no difference in the response between mated and virgin females.

3.8. How does pheromone production vary when males are exposed to females (either direct contact or indirect contact via grain previously infested with females)?

It is known that contact of males with females, or maize previously infested with females, leads to a dramatic decrease in pheromone production. (Smith 1995 unpubl.).

This is thought to be induced by an involatile chemical produced by females called the female factor. An early version of the assay was used to attempt to detect this effect.

This experiment could be repeated using the now improved version of the assay to resolve the timing of this decrease in attractivity of male sources. Grain previously infested with females as well as live females should be tested to control for any effects created by the presence of insects other than the female factor.

3.9 What is the energetic cost of signalling?

Gilson apparatus may be used to indirectly assess the energetic investment of pheromone signalling by males.

4. Determine if there is individual variation in attractivity via pheromone emission between males.

4.1. Do females show significantly different response scores to different males when they are presented individually?

Using the bioassay described in aim three, the response of females to different males of the same age/mating experience could be consecutively assayed.

4.2. Do females show consistent preferences for specific males when given the opportunity to choose between two males?

Choice type assays could be used where females are simultaneously given a choice between two males. In other words, two source pots are connected up to the arena at once (See Wang 1992).

5. Formulate and test more detailed predictions arising from the results of the aims above.

These have yet to be proposed.

RESULTS SO FAR AND SOME DISCUSSION

2a.1. Virgin beetles have been found to mate quite readily even when not in their tunnels (Li 1988 suggested that tunnels are the main site of copulation). Females will mate with several different males in quick succession when placed in a filter paper lined petri dish. One female was mated with 6 males one after the other at which point I ran out of potential suitors! Sperm transfer was later confirmed by the production of viable offspring by these females.

2a.2. Males will successfully mate more than one female within at least 15 minutes delay between matings.

2a.3. This has yet to be investigated.

2a.4. This has yet to be investigated.

2a.5. This has yet to be investigated.

2b.1. Dissection of the female reproductive tract of *P. truncatus* revealed the presence of a sclerotized spermatheca connected to the vagina via a short duct, see Fig. 3. This was also connected to a glandular organ, possibly used to provide nutrients to the sperm.

2b.2. This year work has started in determining the optimum dose of radiation which will effectively sterilise males, yet leave their sperm undamaged to the extent that they will still initially fertilize eggs which cease their development at an early stage.

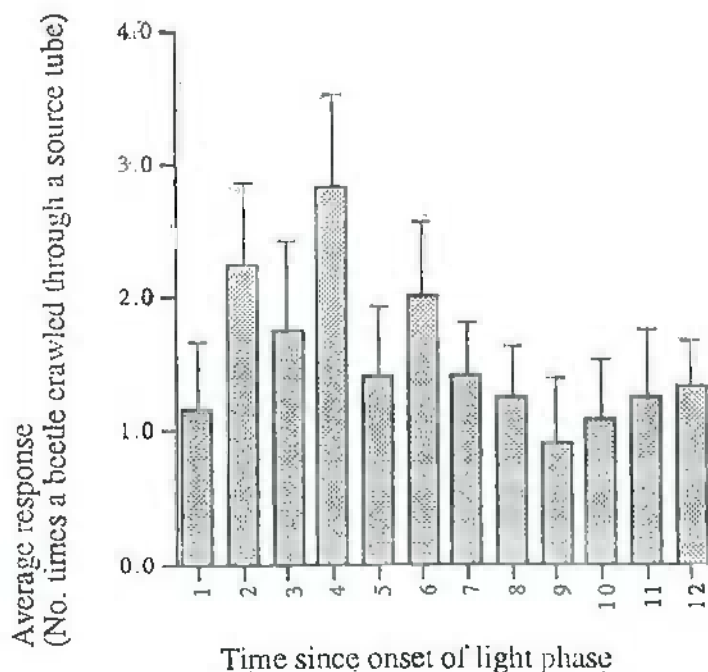
2b.3. This has yet to be investigated.

3.1. Results so far have not detected any conclusive periodicity in combined production and response as measured by this bioassay (see Fig.4). Unfortunately, however, pheromone release from the source pots seems to be smoothed by the influence of pheromone being trapped and subsequently released from the food in these pots as source pots are still attractive after the removal of the male signaller.

The scotophase has yet to be investigated.

Fig. 4.

Graph to show how response to a single male pheromone source varies with the time since the onset of light in an artificial light regime.



Note for all hours considered N=12.

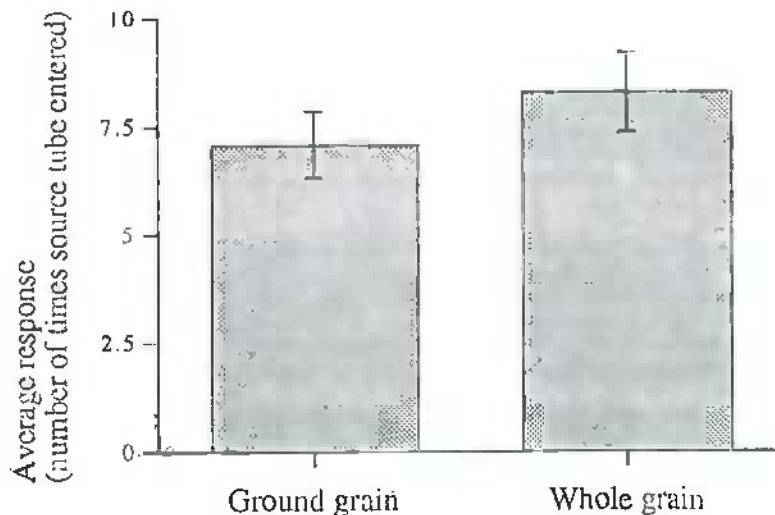
3.2. Pheromone volatiles have been collected for groups of beetles as they age (Smith 1995 unpubl.). This study concluded that pheromone production was likely to be fairly consistent for all ages of beetle with perhaps the exception of very young and the very old. No detectable change in response to sources has been detected in the relatively short time frame of my experiments.

3.3. Females will respond to male sources when they are directly placed in the assay after having been cultured with food, indicating that well fed females are still responsive to pheromone. Females still tunnelling in a grain were also placed in the assay and observed for 2 hours, 11 out of 33 females came out of the grain and oriented to pheromone. This shows that beetles need not be starved to elicit this walking response to pheromone.

3.4. I found that sources set up with ground grain elicited a comparable response to those set up using whole grain, see Fig. 5. This implies that males could attract females to unsuitable oviposition sites if they encountered ground up food, this may be rarely encountered in the field.

Fig. 5.

Bar chart to show average response as measured over two days when sources were cultured on whole or ground grain.



Note N=8 for each treatment.

3.5. Males were found to elicit a response as soon as 20 minutes after being placed in a fresh source pot and no detectable subsequent lowering of induced response was found over the next 7 hours and the next day indicating that this disturbance did not greatly affect the attractive properties of the source.

3.6. Response: In my trials, no loss of response has been detected for responders exposed to pheromone at a rate of one trial per day. This indicates that this rate of exposure does not greatly effect response and in most circumstances females can be used more than once during an experiment.

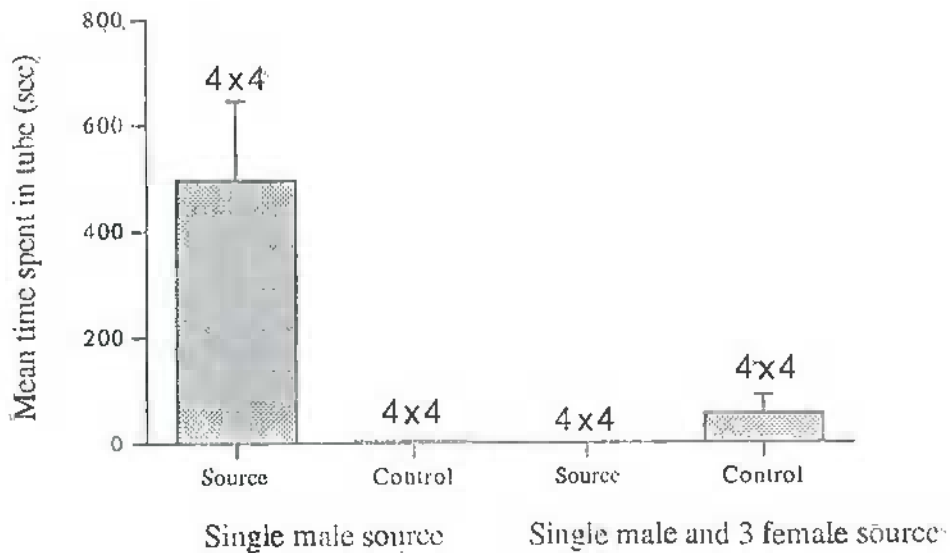
Production: This has yet to be investigated.

3.7. I have performed trials with 33 tunnelling females exposed to single male sources as described in 2C where approximately half were previously given the opportunity to mate. Only 8 were later confirmed as successful matings (by later detection of larvae). Note that the low percentage of matings that were successful is probably a reflection of the beetles used. The virgins used in this experiment were ones left over from other experiments and tended to be of low quality. These preliminary results showed that 12% (n=8) of definitely mated females responded to pheromone compared to 67% (n=25) of those females assumed not to have successfully mated (a group consisting of

females exposed to males and also those not given the opportunity to mate). This data was analysed using a Fisher's exact test (stat=0.018 and $p < 0.02$). This experiment should be repeated with a larger number of better quality females.

3.8. The results are summarized below in Fig. 6.
Fig. 6.

Bar chart to show mean time spent in tube by females exposed to single male sources and sources containing a single male with 3 females.



Note that the numbers above each bar indicate that four different sources were assayed four times.

- 3.9. This has yet to be investigated.
- 4.1. This has yet to be investigated.
- 4.2. This has yet to be investigated.

OVERALL JUSTIFICATION

This study will use a study species of great economic importance to answer questions of broader application. Any evidence concerning the function of this aggregation pheromone may be discussed in terms of other insect signalling systems. As is the case in *Prostephanus truncatus*, many of these systems are of direct economic and ecological importance to man. Perhaps more interestingly, the role of such pheromone signalling in sexual selection will be evaluated. If this pheromone turns out to be a sexually selected character then this work can begin to investigate properties of sexual selection, currently a popular field in modern evolutionary biology.

Fig. 3: Light microscope "squash" slide preparation of part of the female *P. truncatus* reproductive tract showing the spermatheca (Magnification x100, actual diameter of spermatheca approx.= 0.2mm).

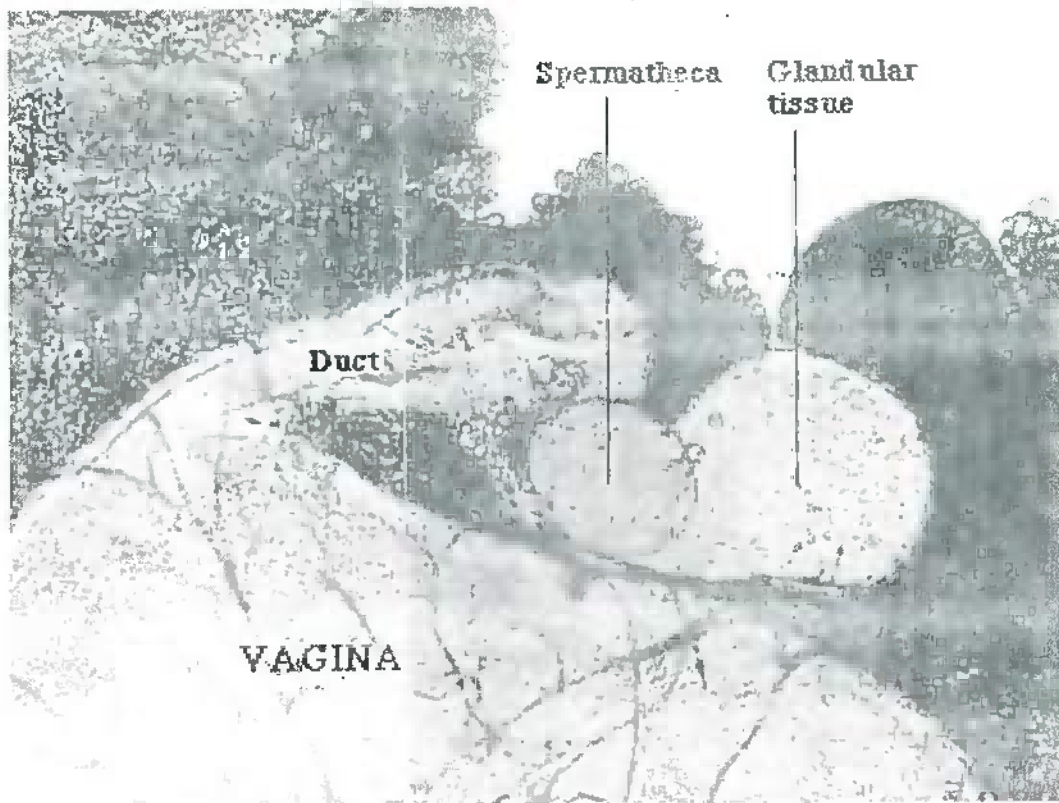


Fig.9: Light microscope "squash" slide preparation of *P. truncatus* male intromittent organ (mag. x100, actual length of intromittent organ approx. 0.6mm).



APPENDIX 1: Details of bioassay

The bioassay is basically a choice chamber with four different air currents leading into a central arena. The assay uses the walking and crawling behaviour of adult females to assess short range attractive properties of volatiles created by males.

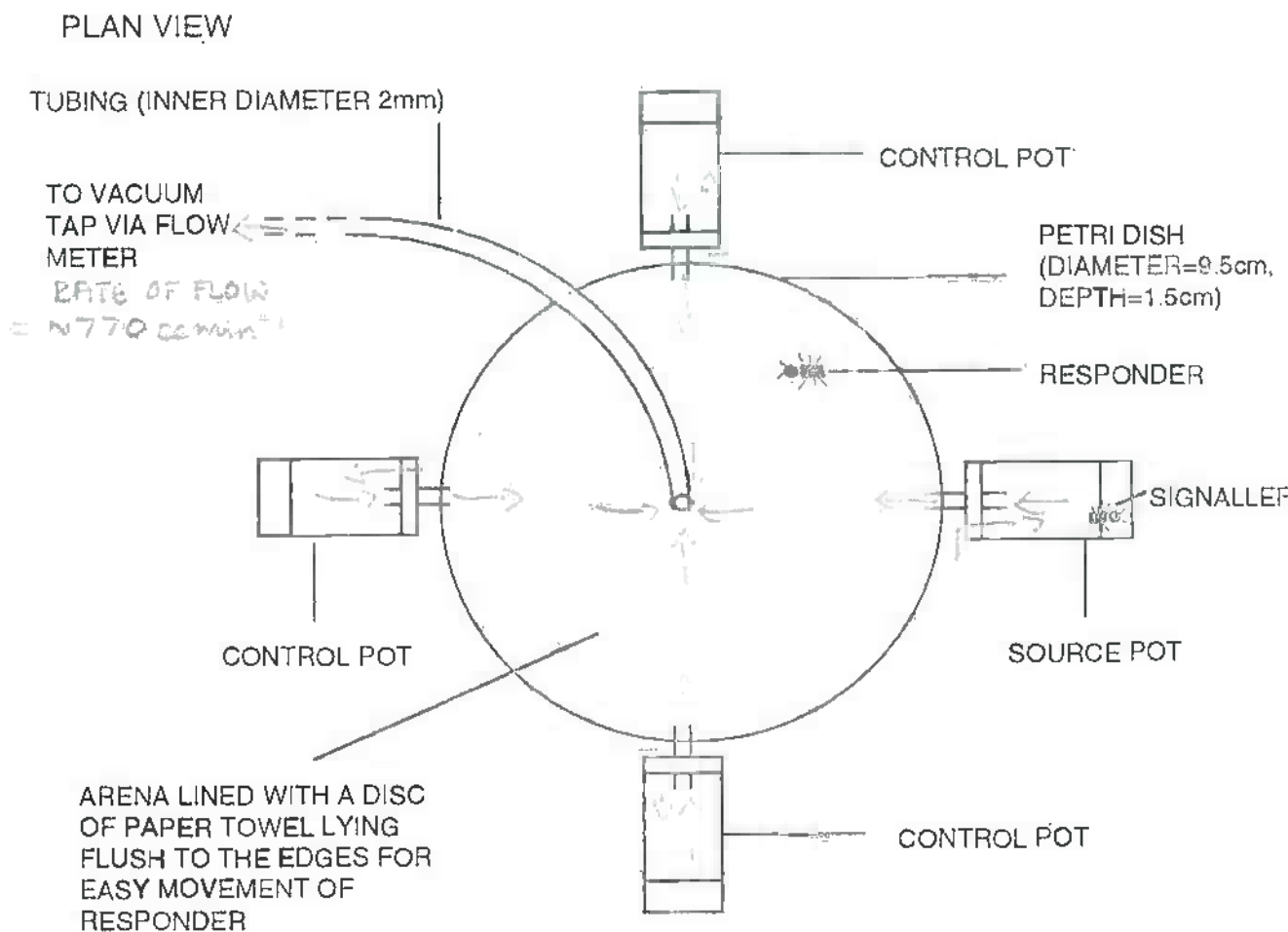
Apparatus: See Fig 3. The petri dish (central arena) has a hole drilled in the lid connected to a vacuum pump which draws air from outside the apparatus through the holes in the lids of the pots and into the arena through clear plastic tubing. Air currents are thus drawn from four pots containing either the beetle source to be assayed, or just grain inside. Grain only pots act as a control for the influence of food volatiles, or the inherent tendency of beetles to crawl down tubes. The petri-dish is lined with a disc of paper towel that is very slightly larger than the base of the dish so it can be pushed in and smoothed out so it is flush and beetles don't crawl under it. This provides a rough surface over which beetles can move easily. All assays are performed in a constant temperature room where temperature, humidity and light regime are largely controlled.

Procedure during the assay:

- The next sources to be assayed are connected directly to suction tube for 5 minutes to remove volatiles already present in air in the container.
- Hands are wiped with IMS (quickly evaporating solvent) before handling fresh tubing or paper discs.
- Petri-dish is wiped with IMS.
- New paper lining is put in the arena.
- New tubes are used to connect up the pots to the arena (control pots are used several times).
- Suction is checked.
- New source pot is incorporated into the apparatus in a random position around the arena.
- A new responder beetle* is placed in the middle of the arena, the lid is put back and the stop watch is started.
- For the next 30 minutes the number of times a responder places it's head and prothorax into each tube is recorded. Each time this occurs the responder is returned to the middle of the arena.
- Assay is dismantled.

*Responder beetles used are females which have been isolated from other beetles, placed on broken up grain, and kept in a separate incubator away from possible pheromone sources for at least 4 days. Females are sexed by looking for bright yellow sac exposed when the adult lifts up the terminal segment of the ventral side of the abdomen. Pressing lightly on the ventral side of the abdomen of a male everts a three pronged intromittent organ (see Fig 9) which can be used to confirm males. Adults are sexed in this way rather than just looking at the clypeal tubercles (Shires 1980) because 100% certainty is needed. Using this method ensures that no males get into the "pheromone free" incubator and allows responders to become more sensitive to pheromone.

FIG. 2: DIAGRAM OF ASSAY APPARATUS.

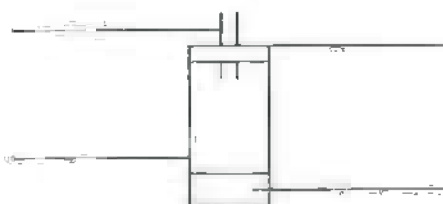


→ = AIR FLOW

DETAIL OF POTS

CLEAR PLASTIC TUBING OF (INNER DIAMETER=2mm) CONNECTING CONTAINER TO ARENA

GLASS CONTAINER



PLASTIC LID WITH ADDITIONAL HOLE TO ALLOW AIR INTO CONTAINER

FOOD SOURCE

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APPENDIX 2

AIM 1: COLEOPTERAN AGGREGATION PHEROMONES

Communication between organisms takes many forms. From a human perspective watching and listening to others are perhaps the most obvious ways in which we receive signals. For insects, the subject of this review, the world of smells/tastes has heightened importance and can relay surprisingly precise information. Karlson and Butenandt proposed the term "pheromone" in 1959 to describe, "substances which are secreted to the outside by an individual and received by a second individual of the same species in which they release a specific reaction". This definition serves to distinguish pheromones from hormones which are chemicals conveying information within the body of a single organism, and also from kairomones (attracting exploiters) and allomones (attracting organisms of benefit) which are chemical messages, intentional or otherwise between individuals of different species. However, these definitions are in no way mutually exclusive and the same chemical may be acting in more than one capacity at any one time depending on who you are considering. Semiochemicals is a more general term encompassing pheromones kairomones and allomones. Bossert and Wilson classified pheromones further in 1963 into 'releaser' substances and 'primer' substances. Releaser substances are those which induce an immediate and reversible change in the recipient acting more or less directly on the central nervous system, and primer substances are those which trigger a more permanent physical change in the recipient, for example retarding sexual maturity. In this review I will be dealing with releaser pheromones, more specifically, "substances produced by members of either or both sexes that induce members of both sexes to aggregate", termed aggregation pheromones (Borden quoted in Kerkut 1985). These are superficially distinct from sex pheromones which attract just the opposite sex from that of the signaller.

The "problem" to be discussed is "what is the function of aggregation pheromones?". Both adaptive and non-adaptive explanations are possible, and these explanations may be very different for those who are signalling and those who are responding to the signals. It is tempting to look on the scale of the group when looking for adaptive explanations for the formation of aggregations, after all, it is a phenomenon involving many individuals (see Shorey 1973). Explanations like, "...because the food resource may be either ephemeral or not available until the tree dies, these beetles have evolved a pheromone that elicits behaviour resulting in aggregation of the population on the new host..." are put forward (Wood 1982). Here I want to propose that potential explanations should be described in terms of individuals to avoid wrongly categorising such explanations as group selectionist. Therefore I would start rephrasing Wood's proposal to read, "...a beetle which locates a host might benefit from advertising its location because....", followed by, "beetles may chose to move towards/away from this signal because....". Notice how the signaller cannot necessarily dictate the response of the receivers of the signal. The signaller cannot force other beetles to be attracted or repelled, they will, assuming they are responding optimally, behave in which ever way optimises their fitness. Alcock (1982) has led the way in demanding these kind of explanations as current evolutionary thinking dictates. Alcock considered the bark beetles, here I attempt to review aggregation pheromone systems in these and additional families and in doing so propose new explanations for the patterns of pheromone production so far found in Coleoptera. The literature on Coleopteran aggregation pheromones is composed mainly of various insect pest species. The impact created by such aggregations has been the inspiration behind much of the funding given over to this subject. For this reason, it is to these insects, the weevils (Curculionidae), the grain borers (Bostrychidae), the flour beetles (Tenebrionidae), the sap beetles (Nitidulidae), the Chrysomelidae, the flat bark beetles (Cucujidae) and the well studied bark beetles (Scolytidae) that this review has searched for clues.

Firstly I will describe some of the general characteristics of the aggregation pheromones reviewed. I will then discuss some of the possible selection pressures and restraints which could have resulted in such an aggregating pheromone system. During this discussion I will use evidence both from the general patterns already described and

more specific data so far gathered which is applicable to this topic to make a preliminary assessment of which functions are best supported at this time.

GENERAL CHARACTERISTICS OF REVIEWED AGGREGATION PHEROMONES

Pheromone produced only when signaller is on food source/suitable oviposition site

Aggregations of insects often form away from an appropriate food source, usually for the purpose of mating. Alternative localities including potential adult emergence sites, potential oviposition sites and just conspicuous features of the landscape like hill tops. However these aggregations are not generally formed by pheromones that attract both sexes and more specifically all the aggregation pheromones reviewed here are produced in the presence of an appropriate food source. The necessity of this stimulus has been specifically demonstrated in Chrysomelidae (Peng 1992), Nitidulidae (Bartelt 1994) Bostrychidae (Mayhew 1994) and Scolytidae (Vite 1968).

With the exception of Peng 1992 these studies all assess pheromone production directly by collection of volatiles and not via the response of responders. This response may be influenced by the presence of food directly as well as it's effect on pheromone production (see next sub-heading). Peng 1992 controlled for this effect by separating potential producers from the food source with a screen. Faustini 1982 and Rochat 1991a have obtained similar results in their studies in Curculionidae but without such a control being included.

Many monoterpenes are toxic to the beetles that feed on them and it has been proposed that the biochemical pathways which produce these pheromones may have initially evolved as part of a detoxification process. The detoxification strategy generally being geared to converting hydrophobic structures into hydrophilic ones which are easier to eliminate. Various isomers of verbenol and verbenone and myrtenol are derived from the tree oleoresin component alpha-pinene in bark beetles. Myrtenol, another derivative of alpha-pinene is known to be produced by *Ips paraconfusus* and *Dendroctonus frontalis*, *Dendroctonus ponderosae*, and *Dryocoetes confusus*. Although myrtenol has so far no known pheromonal function in *Ips* species, it is a multifunctional pheromone of *Dendroctonus frontalis* and an aggregation pheromone of *Dryocoetes confusus* (Discussed in Prestwich 1987).

Even the distinction insinuated between host odours and insect produced pheromones becomes less obvious since many pheromones are derived more or less directly from host chemicals ingested by a feeding insect. At the other extreme, pheromones may be assembled from much more basic compounds by the signaller. This has been demonstrated to be possible for at least two Coleopteran species so far. Boll weevils were shown by Mitlin and Hedin (1974) (quoted in Prestwich 1987) to incorporate radio labelled acetate, mevalonate and D-glucose into all four components of grandlure. Similarly, *C. ferrugineus* was shown to use both acetate and mevalonate for the construction of a terpenoid pheromone (quoted in Prestwich 1987). However, both these species can also derive these pheromones more directly from dietary components. Where the pheromone is derived from the host plant chemistry, presence of the host is obviously required for pheromone production. Interestingly, the host plant also seems to be required before production of aggregation pheromone even when the pheromone is truly being synthesised by the insect.

Response to pheromone is often heightened by the confirmation of the presence of food via perception of food volatiles

Potential responders to a pheromone signal may assess the existence of a food source before responding by using host volatiles. The importance of the pheromone signal to be in the context of such volatiles varies from very strong synergism such as that found in Nitidulid beetles (Bartelt 1993a) to as yet no detectable influence (Pierce 1995 for

Conophthorus species of Scolytidae, but see enhancing effect noted by Birgersson 1995). Bartelt 1993a found that pheromone alone attracts only 1-29% as many beetles as the combinations with dough (source of food volatile), and dough alone only 0-2.9% as many as in combination. Further studies commenting on these synergistic effects in Nitidulid beetles include (Bartelt 1992, 1993b, 1994, and 1995, Blumberg 1993, Dowd 1991, James 1993 and 1994, Lin 1992 and Williams 1993). Here it is volatiles produced by the action of yeast on the host rather than volatiles emitted by a host alone which seem to be most influential. Other synergistic influences of pheromone/food volatile combinations have been described in the Curculionidae (Walgenbach 1986, Weissling 1994, Giblindavis 1994, Gries 1994b, Rochat 1993a and Phillips 1993) and Scolytidae (Pitman 1969, Byers 1990). Other patterns of influence include additive effects (Renwick 1969) and density dependent but ill defined effects (Dowdy 1993), and Jaffe 1993 proposes an interaction which is distance dependent. Petroski 1995 propose that the pheromone and co-attractant bind in close proximity on the receptor surface. This evidence comes from molecular modelling of 26 host type volatiles with pheromone from Nitidulidae. The use of host volatiles in combination with pheromone components in traps is reviewed in Faustini 1990.

All the food sources reviewed also double as sites of oviposition, perhaps their more important role since often more of the host is consumed by the developing larvae than by the mature adults.

The food source is capable of supporting many individuals

Here the data available is, unfortunately biased towards studies on insect pest species. Therefore food sources are often artificially large, a good example being a grain store. However, I have found no studies which describe beetle species that produce aggregation pheromones which generally feed on small patches of food. However, such situations could not result in the large numbers of insects which tend to stimulate research funding so the possibility of this occurring cannot be ignored.

The identity of the signaller is sex specific

Both males and females have been found to produce aggregation pheromones but for any one species there is always (as far as I have found) a sex specific pattern of pheromone production. The signallers may even show greater response to host volatiles (Gast 1993). Male produced aggregation pheromones have been described in Bostrychidae (Dendy 1991 and Khorramsh-ahi 1981), Cucujidae (White 1989), Curculionidae (Budenberg 1993, Evans 1994, Faustini 1982, Hibbard 1993, Jaffe 1993, Nielsen 1993, Patrock 1992, Perez 1994, Rochat 1991a and 1993, Walgenbach 1983, Weissling 1994), Nitidulidae (Bartelt 1992, 1993b, Petroski 1994), Tenebrionidae (Obengofori 1990a and b), Scarabae (Gries 1994a) and some genus of Scolytidae including *Ips* (Akers 1993, Borden 1967, Gast 1993, Miller 1991, Seybold 1995, Byers 1990, Teale 1991, Wood 1982, Zuber 1993), *Polygraphus* (Bowers 1990, Francke 1983), *Dryocetes* (Camacho 1994) and *Pitogenes* (Byers 1993). Females have been found as the initial signallers in several genus of Scolytidae including the *Dendroctonus* (Agosta 1992, Wood 1982, Pitman 1969, Renwick 1969), and *Scolytus* (Peacock 1971? see library). Mostly only one sex produces. Where both sexes signal it is mostly with different chemicals. Cases where both sexes signal with the same chemicals are rare and in the case of the Cucujid beetle, *Ahasverus advena* the rate of production differs with males producing at least 4 times as much as females (Pierce 1991).

The identity of the responder is not sex specific

By definition aggregation pheromones attract both males and females. Also in many cases the sexes arrive in equal proportions (Field studies: Patrock 1992, Peng 1992, Laboratory studies: Obengofori 1990a, Dowdy 1993 and Walgenbach 1983). However this is not always the case and there are many cases of sex specific differences in the pattern of arrival. Sex specific patterns of response can arise from many variables and it is important to realise the limitations of data collected from the field. Differences in trap

catch number between the sexes can by no means be attributed to differential sensitivity to attractant. The sex ratio of beetles dispersing is often not equal. Zuber (1992) found a higher proportion of males in traps at the beginning of a flight period in *Ips typographus* thought to arise from sex specific patterns of dispersal. A similar situation was found by Chapman in 1966 for *Typodendron lineatum* and Rudinski (1963) for *Dendroctonus pseudotsugae* where additionally females numbers peaked late in the season in synchrony with reemergence for "second brood" formation. *Ips typographus* and *I. pini* response to pheromone was found to be affected by nutritional state (Nemec (1993 and Gast 1993 respectively). Mating status can also affect response (see next sub-heading). Thus the timing of trap sampling can greatly influence the result obtained. *Polygraphus rufipennis* showed no sex-specific catch differences in the Spring and Summer, however more of the opposite sex to the signaller were caught in Winter (Bowers 1990). Teale 1991a found a seasonal variation in response to ipsdienol by *Ips pini*. Laboratory bioassays are usually designed so that many of these confounding variables are controlled for.

Where there is a difference in response between the sexes, usually it is the opposite sex to the signaller that shows the greater response (Field data: Weissling 1994, Krausseopatz 1995 Laboratory data: Weissling 1994, Evans 1994, Faustini 1982, Bowers 1990). The results obtained by Obengofori 1990a for *Tribolium castaneum* where male pheromone attracted more males than females is the exception. Sex specific differences have also been found at the level of pheromone perception. Chambers 1990b found females of the Cucujid beetles, *Cryptolestes ferrugineus* and *C. pusillus* had larger EAGs than males when exposed to the male produced pheromone components. EAG results are just one level of influence on behaviour and studies by White (1989) show marked sex-specific behavioural differences where no differences in EAG patterns were found. Faucheux (1994) has even found structural differences between the sexes with respect to their sensilla cells.

Discrepancies between the response of the sexes can be further complicated when different components of pheromone blends skew the sex ratio in different directions. As we find out more about pheromone systems it is likely that more components will be revealed and an ever more complex pattern of response will become apparent. *Ips pini*, a particularly well studied species of bark beetle appears to show such a pattern. Gast 1993 found a greater percentage of females (58%) would respond to the male produced pheromone component Ipsdienol than males (36%). Teale 1991 had already found that as the proportion of a second male produced component, Lanierone, was experimentally decreased, the total number of beetles caught increased and the proportion of those that were males decreased. Teale has taken this further and in 1994 it was shown that female preference was actually correlated to the blend of enantiomers of ipsdienol produced by individual males. In another Scolytidae, *Dendroctonus brevicornis*, at least one attractive pheromone component is produced by each sex which preferentially attracts the opposite sex. Combined, the blend results in an approximately equal sex ratio (Agosta 1992). The Saw toothed grain borer, *Oryzaephilus surinamensis* has a multi-component pheromone which White (1989) perhaps controversially proposes allows males to alter the relative response of the sexes to the aggregation pheromone. In the Tenebrionidae, *Tribolium castaneum* shows sex specific differences of response that can be altered by changing the ratio of the components (Rangaswamy 1991a). As well as blends of many components, the enantiomeric ratio of single components can affect response sex specifically (Salom 1992). The ratio of pheromone to host volatiles may also influence the sex ratio of beetles attracted. In *Dendroctonus ponderosae* low trans-verbenol:resin ratio attracts mainly females where a high ratio results in a male bias (Quoted in Raffa 1993 and considers sex specific responses of Scolytidae).

Effect of mating on production of and response to pheromone

Production of pheromone has been shown to change in correlation with the presence of females and/or mating experience. This is not always the case and as yet no effect has been demonstrated in the Bostrychid, *Rhyzopertha dominica*, (Dowdy 1993 and

Mayhew 1994). However, for a related species, *Prostephanus truncatus*, pheromone production by the male can be decreased dramatically by exposing the signaler to females, even if indirectly in the form of grain previously infested by females (Smith 1995 unpubl.). Both *Polygraphus rufipennis* and *Ips confusus* males showed decreased pheromone production in the presence of females (Bowers 1991 and Borden 1967 respectively) with production decreasing in direct correlation with the number of females per male in the case of *I. confusus* (see later discussion). Chapman's studies on *Trypodendron lineatum* found log attractiveness to decrease rapidly on addition of males and concluded that female pheromone production was suppressed on mating (Chapman 1966) The same pattern was found to be the case for another Scolytid, *Dendroctonus pseudotsugae* (Rudinski 1963). Mating was not found to effect pheromone output by males of the maize weevil, *Sitophilus zeamais* (Walgenbach 1983).

Mating status is also correlated to the level of response to pheromone. Quoting from Borden 1967 when discussing *Ips confusus*, "reproducing females show a greatly reduced response to male attractant". Walgenbach (1983) found that only the female's response to pheromone decreased with mating and the male's response remained high whatever the mating status. No differences have been found between the responses of virgins versus non-virgins in Bostrychidae (Obeng-Ofori 1990a, Dowdy 1993), Tenebrionidae (Obengofori 1990a) and RoCHAT (1991a) found no obvious effect for the American palm weevil, *Rhynchophorus palmarum*. Gast (1993) noted a more general pattern where gonad development in adults was significantly correlated to response to pheromone and so "proportionately more responders were sexually mature than in the total population". Of course as with any correlation this does not in itself imply a sexual function to the pheromone. The result could simply arise if sexually mature beetles were more likely to be dispersing and it is this broad category of beetles that is preferentially attracted.

Anti-aggregation pheromones

The growth of aggregations is often limited at least to some degree by repellent pheromone signals and/or the cessation of signalling. Repellents can be distinct chemicals from the original pheromone or different blends, or concentrations of chemicals that in another format, are attractive. The blend of endo and exo-brevicommin determines whether it is attractive or repellent to responders of *Dryocetes confusus* (Camacho 1994).

Unlike the attracting pheromones, aggregation inhibitors are usually produced by both sexes. For example both males and females of *Dendroctonus brevicomis* (Bertram 1994a) and *D. frontalis* (Payne 1992) emit verbenone, a common aggregation inhibitor in Scolytidae. 3,2-MCH has inhibitory effects for *D. pseudotsugae* and is produced by both sexes (Wood 1982 and Ross 1994). Both males and females of *Ips paraconfusus* produce cis-verbenol (Wood 1982), which acts as an inhibitor of male produced aggregation pheromone components (Akers 1993). Anti-aggregation pheromones are generally much less species specific than their corresponding attractants. As already mentioned verbenone is common amongst the Scolytidae, as is Ipsdienol (references above and Borden 1992, Safranyik 1992, Shore 1992, Paine 1991, Devlin 1994, Miller 1992, Byers 1993). It is proposed that the higher generality of these compounds between species allows them to function to limit both inter as well as intra-specific competition. Indeed the pheromone of one species often has an inhibitory effect on other sympatric species (Schlyter 1993, Miller 1992, Byers 1993).

Anti aggregation compounds have obvious potential to be applied to pest management. They can be used to disrupt colonisation (Payne 1992) and they are often used in conjunction with attractants in a "push-pull" strategy which can concentrate the pest away from high value commodities (Ross 1994, Smart 1994)

The trigger for anti-aggregants is thought to be a function of population density (Agosta 1992). It is also often found that the production of attractive pheromone per beetle decreases with the number of signallers in an aggregation. For instance, Australian sap beetle males in groups of 50-60 emitted a peak level of 0.09 μ g per beetle per day compared with a single male emission rate of over 3 μ g per beetle per day (Bartelt 1994). Amount of pheromone per male decreased when population density was artificially manipulated in the Bostrychid *Rhyzopertha dominica*, however in this study mixed cultures were used (Dowdy 1993 and Mayhew 1994). Pierce (1991) found that pheromone production of the Cucujidae, *Ahasverus advena* was barely detectable in the highest population densities of mixed sex cultures tested. Unfortunately it would be very difficult, if not impossible to monitor the breakdown of pheromone production across many co-habiting individuals. It is not known whether such decreases in pheromone production arise from some individuals completely ceasing production or a more general lowering of production by all individuals. In common with attractants, response to repellents can vary with their release rate (Bertram 1994b, Miller 1992), the season (Devlin 1994), and many other influences.

POTENTIAL EXPLANATIONS TO ACCOUNT FOR THE OBSERVATIONS ABOVE.

Potential explanations for the existence of aggregation pheromones are obviously governed by many assumptions and limitations. Looking from the signaller's point of view for instance, is the production of pheromone a substantial cost, or very little cost, costly not to produce, or indeed unavoidably produced? The answer to this point will depend on the source of pheromone. If the pheromone is synthesised de novo as is indicated to be the case in some beetles studied (Seybold 1995, Ivarsson 1993), then production is likely to be a cost of some description, and indeed Schlyter (quoted in Schlyter 1989) estimates that male *Ips typographus* expends the equivalent of 2% of its body weight per day when emitting pheromone. On the other hand, many pheromones, as already mentioned are derived from host compounds and maybe waste products of digestion. To eliminate the emission of such compounds following feeding may represent a significant cost to the signaller (Renwick and Hughes 1975 quoted in Alcock 1982) and in this case benefits to the signaller may not be needed to explain such signalling behaviour. Indeed the signaller may, on balance lose by signalling if the cost of not signalling is even higher.

Costs of signalling are not limited to energetic costs, the signal may create costs from it's influence on other species. Potential competitors and/or predators can be attracted. Predators from a wide range of taxa have been shown to attracted (Prestwich 1987, Wood 1968, Schulz 1987, Bowers 1992) and may represent a selection pressure capable of changing the fine detail of pheromone structure/composition. *Ips pini* varies the enantiomeric blend of ipsdienol from place to place and from year to year, this corresponded to a change in response of its predator *Thanasimus dubius* and possibly represents predator/prey co-evolution in action (Herms 1991). The possibility of such a system leading to polymorphism in respect to pheromone production characteristics has been modelled for the European corn borer moth (Guldbrandtsen 1995). Interestingly in this case just one-locus seems to determine pheromone blend in females which can be traced using simple mendelian genetics.

Potential benefits of attracting con specifics include better defending capacity against predators, overcoming the resistance of a potential host, and mate attraction.

Defence against predators has been proposed as an adaptive advantage of aggregating in the aposematically coloured beetle, *Lycus toripes* (Eisner and Kafatos 1962).

Collective overcoming of host defences is a convincing function of aggregation in some species of bark beetles, particularly those which attack healthy trees (Wood 1982). Here, although the pioneering beetle has located a potential host it is proposed that it

will either not be able to feed and/or oviposit efficiently until the host is transformed or "conditioned" by the combined action of many beetles. Such a "conditioning" benefit is often inferred, but rarely has it been demonstrated directly. Raffa and Berryman have performed such a study on *Dendroctonus ponderosae* (quoted in Alcock 1982). Evidence that conditioning forms at least some of the motivation behind signalling was found in an early study on *Dendroctonus ponderosa* where a higher rate of pheromone production was observed when females were introduced to logs with higher oleoresin content in exudent (a method of host defence) (Vite 1968).

Conditioning may involve the inoculation of the host with fungal pathogens as is the case in some Scolytidae including *Dendroctonus pseudotsugae*. *Scolytus multistriatus* is thought to inoculate the tree with pathogens e.g., Dutch Elm disease (Wood 1982). Conditioning can simply involve the mechanical action of many beetles. "Mass attack", the situation where an aggregation forms very rapidly could be some kind of method of killing a tree. Alternatively, signalling may be an incidental product of feeding, and the "mass attack" often observed may simply arise as individuals race to take advantage of a comparatively short window where the host is relatively safe in terms of lowered defences and yet still not totally used up by those beetles already present (see Alcock 1982). Although some Scolytid beetles are capable of attacking healthy trees, these are usually only colonised when stressed or dying trees are unavailable, and the majority of species will only colonise weakened or even dead hosts (Wood 1982). *Dendroctonus ponderosa* and *D. brevicornis* can attack healthy trees but beetles have been found to be preferentially attracted to stressed trees (Wood 1982). The Palmetto weevil, *Rhynchophorus cruentatus* is specifically attracted to volatiles from moribund palms, it's host (Giblin Davis 1994).

In the stored product environment where the host resource is commonly made up of much smaller units of food, grain for instance, conditioning could take the form of changed humidity and substrate consistency from the action of many beetles. Beetles could potentially share the cost of burrowing through hard seed coats and use common entrances into a host. However, the benefit of this must be limited by the size of many grains inhabited by these beetles. A single ovipositing female may utilise more than one, and not just part of one grain. For example in the grain horer, *Prostephanus truncatus* a female will distribute her eggs on average in 12.5 grains (s.e. 4.17, n=30) when ovipositing alone (Li 1988). However, Fadamiro (1995) still proposes that the individual cost of boring can be decreased by feeding with more con-specifics. Walgenbach et al looked at *Sitophilus zeamais* aggregations and proposed that chewing through the seed coat of a grain kernel required much energy since females require some 30 minutes or more to make a hole in a wheat kernel large enough to deposit an egg (Walgenbach 1983). They observed that when a weevil was feeding on a fresh kernel of wheat one or more companions were often present and all the grain was consumed before a new grain was burrowed into. I have yet to find a study which specifically looks at the division of cost of burrowing between individuals and obviously if a beetle does not defend the food resource it has created access to there is potential here for exploitation by con-specifics.

Another potential benefit of high adult numbers could be increased offspring survival from a general increase in the amount of food found as dust/flour outside the grains. The survival of juveniles may be largely determined by the environment which they find outside the grain. Offspring of species who oviposit within grains like the Larger grain horer may show particularly high larval mortality if they happen to leave the grain whilst tunneling for food. For this species mortality incurred for this reason may be expected to be highest when the adults are ovipositing on smaller grains since the larvae are more likely to fall out (suggested by Li 1988). Li has found no evidence for such a collective conditioning benefit in this context for the larger grain borer. She found that the number offspring surviving to emergence increased with a gradient of less than one as the number of parents increased ($R=0.98$ for sorghum and 0.93 for wheat). More specifically, combining data from both stored products, the number of offspring

emerging vs the weight of flour made by the parents gave a gradient of $R=0.96$. This species actually breeds best on maize (Li 1988), and for initial adult densities ranging from 6-26 pairs per 60g of maize the average number of offspring per female emerging was never found to be higher for an increase in adult density.

Thirdly, aggregation pheromones may be being used as sex attractants. Some of the general patterns of aggregation pheromones discussed above indirectly support this hypothesis. Sex specificity of production, some sex specificity of response, short term production decline in the presence of mates/after mating all indicate at least some sexual function. Alternative explanations are possible of course, Fadamiro (1995) has put forward the idea that male signalers use the presence of females, detected via an involatile chemical, as an indication of population density, and thus the observed decrease in pheromone production on exposure to this chemical need not be explained in terms of any mating function. Males disperse slightly more readily than females and so the detection of a female may be used as a cue that sufficient beetles have arrived to confer a collective feeding benefit.

If aggregation pheromones are primarily sex attractants then the obvious question is why, unlike conventional sex attractants, do aggregation pheromones attract both sexes? Even though the motivation behind this message may be to attract potential mates only, once sent the signal is available to all who can detect it. In the case of male produced aggregation pheromones, females may orientate towards it because they can predict that it is the location of a mate / food source / oviposition site. Males may also orientate towards it since they could predict that it is the location of a suitable food source / site where females are likely to aggregate. Therefore, where the signaler attracts conspecifics of the same sex, the signal may incur a cost of increased competition for resources specifically mates. This is thought to be the situation found in some field crickets described in Alcock (1982) who signal by chirping.

Males may be most likely to respond to a signal by conspecific males if the species usually utilises a host which can potentially support many individuals and he can expect to find many potential mates aggregated on a large food/oviposition resource. If potential hosts are few and far between he may have no choice but to follow the signal in order to stand any chance of leaving offspring. If potential hosts are common then more male/male competition may occur at the signalling stage with many males releasing signals at once. In this situation it is possible that attracting some conspecific males may be an advantage. If the collective signalling of greater numbers of males attracts more females per male then it might be possible for this behaviour to be adaptive. Pheromone emission per beetle often decreases with the number of signalers available (see previous discussion). This is compatible with the idea that beetles may gain in decreased energy costs by synchronised signalling. However, if paternity is not split equally within the aggregation some males may reduce their reproductive fitness by attracting extra male conspecifics.

Males are unlikely to signal if they are not on a suitable host since even if the attracted female will mate, there will be a delay whilst she then locates a suitable host which may decrease the survivorship of these offspring. Dishonest signals may only be expected where potential hosts are common and paternity is biased towards the 1st mated male in situations of multiple copulation. After all, the longer the delay between mating (which is inevitable if a male signals when not at a suitable host) the higher the chance that the female will remate with other males before laying. The use of host volatiles as part of the signalling system (discussed above) may also limit dishonest signalling.

How could selection pressures change as the aggregation grows? Here I will consider the situation where the original signaler is male or female and where the host quality is initially improved by additional beetles (Host type 1) and the case where the host condition is around an optimum for the initial coloniser (Host type 2). In Figure 7 I have predicted the general pattern of major components of fitness for males and females!

as an aggregation develops on the two types of host. Therefore the x-axis represents both an increase in numbers of individuals in the population on the host and a progression of time. How these two variables are correlated is not considered here. Note an equal sex ratio is assumed for simplicity.

In the first graphs 7a and 7b I consider how population size might limit the number of offspring male and females might produce/father. For females the number of mates available may only be expected to limit the number of offspring produced at very low numbers, the presence of just 1 male may be sufficient. Increasing numbers of males may lead to a better quality of offspring as population numbers increase if male-male competition is encouraged at any level. For males the average number of offspring fathered must be equal to that of the females, however, the division of those offspring between potential males will change as population size changes. The exact pattern of paternity will depend on the level and mechanisms inter and intra sexual selection. Assuming this to be non-random, the potential maximum number of offspring fathered by the most successful males could continue to increase for longer as the population rises than is the case for females or for males of low competing ability (see graphs a and b). The max. number of offspring fathered plateaux will only be reached when the male becomes sperm/time/energy limited. For the less successful males represented by the lowest line on this graph, no further gain in offspring number fathered is likely to be predicted as population size increases.

Benefit from signalling may undulate quite rapidly for any single signaller. As mates arrive pheromone production may be cut off for a while until the signaller is free to remate again. The number of matings optimal for any individual will obviously vary both between the sexes and between species. Pheromone production should really only be sustained for polygamous males with low investment per female. Polygamous males of the maize weevil, *Sitophilus zeamais* do not show decreased pheromone production with mating (Walgenbach 1983). High investing males, like *Ips confusus*, who construct the nuptial chamber and actually guard this against intruders of both sexes, might be expected to decrease pheromone production as he acquires the optimum number of mates he can support. This was found to be the case (Borden 1967). See discussion above for cases where female pheromone production declines with mating.

Overall reproductive fitness is a product of the number of offspring produced and their survivorship. In graphs 7c and 7d I consider the limit of resource availability (both food and oviposition sites), and how it is influenced by population size assuming that the size of the host is finite and non-renewable. In graph 7c the host resources are not available until a certain population size is reached as proposed in the collective conditioning hypothesis described above. In graph 7d host resources are available to the 1st coloniser and do not increase in quality as the population increases. In both graphs the decline in survivorship at high population numbers represents the point where the host resources start to run out. Zhang (1992) has demonstrated such changes in life history traits at high population densities for *Ips cembrae*.

This model predicts that a signal likely to result in an aggregation of both sexes would be of most adaptive value to both males and females who have located a type 1 host and males who have located a type 2 host. This variation in potential benefit from signalling will only translate into an observable difference in propensity to signal if there is a real cost to signalling/host finding. Interestingly in the less common situation where the pheromone is produced by the female these species are often those which colonise type 2 hosts. Specifically the Dendroctonus species of Scolytidae, many of whom are known to kill healthy trees and require this for successful egg laying (Ref. Included in this category are also species of the scolytus genus like the tree killing species, *Scolytus multistriatus* and *Scolytus ventralis* see Wood 1982). In the Scolytid *Trypodendron lineatum* the female produces the pheromone, yet this species is not considered to be particularly aggressive and is not known to actively kill trees. This species is also one of where pheromone production ceases once mated implying a sexual function.

The second prediction which depends on the prerequisite of considerable non-randomness of mating is that actions leading to an increase in local population size may have different fitness consequences for different quality males, therefore those that represent the max. line in graph 7b would gain more from initiating an aggregation than those representing the min line. It is questionable whether this predicted variation in potential benefit from signalling would transcribe into observable differences in investment into host location and/or signalling. A trend may only be observed if 1) Considerable variation in success in direct male-male competition exists, 2) Traits such as investment in host location and/or signalling are phenotypically plastic or alternatively, ability to do well in male-male competition and level of investment in signalling is genetically linked and 3) If there is a cost to signalling such that if a male with low competitive ability can maximise his fitness by saving resources on signalling and host location and investing in orientating to other male's pheromone signals. Variation in pheromone production certainly exists in the Boll weevil where selective breeding for it's increase has led to a 4.5 fold increase in selected strains as compared to the parent population (McCoy 1990).

If mate acquisition is the main benefit incurred from signalling then we might predict that characteristics of pheromone production, particularly where the signaller is male, may be shaped by sexual selection. In other words, females may choose between males on the basis of their pheromone signal. Division of paternity between competing males could be influenced by variation in signalling, behaviour when in contact with a female or other males, and sperm competition. The relative importance of these characters may vary greatly between species. What evidence have we already which is compatible with the idea that females use pheromone production to choose between males? Male olfactory attractiveness to females is positively correlated with a male's subsequent fertilisation success as measured by it's p_2 value in double matings in the red flour beetle, *Tribolium castaneum* (Lewis 1994). So in this case two possible influences on the pattern of paternity are correlated. The third predicted influence may be of limited importance in this species as, "neither males nor females exhibit aggressive behaviour towards members of their own or the opposite sex" (Lewis 1994). Assortative mating has been correlated to pheromone characteristics in *Ips pini* where it is the ratio of enantiomers of ipsdienol which was found to be variable Teale (1994). Females were found to have a consistent preference within the variation of ratios and males were found to show highest response to blends similar to those that they were subsequently found to produce. Where different pheromones are used for short and long range signalling it may be predicted that the shorter range components will be most influenced by sexual selection since it is at this point that a female is most likely to encounter more than one signal and has most opportunity to make comparisons.

Thirdly, as the aggregation grows, motivation to signal should decrease and eventually cease (at the arrows in Fig. 8.). Beyond this point there may be selection pressure to signal to conspecifics that the host is saturated (see anti-aggregation pheromones). Evidence of intraspecific competition at higher beetle densities has been found in *Dendroctonus ponderosa* where there is a strong negative correlation between density of attacks in cut (i.e. defenceless) logs and pupal production per female" (Raffa and Berryman (in prep) quoted in Alcock 1982). Raffa (1993), suggests that "inhibitory pheromones may function as "pre-rivalry" signals in male-male interactions, in that both signaler and responder benefit from avoiding rivalry" (see also Pierce 1995 and Miller 1992). The honesty of such signals may be ensured if response to them is dependent on their concentration and this is a truly constrained by the number of beetles signalling. Dose dependent inhibition of attractant by verbenone has been found in three Scolytid beetles where higher doses give most inhibition (Miller 1995).

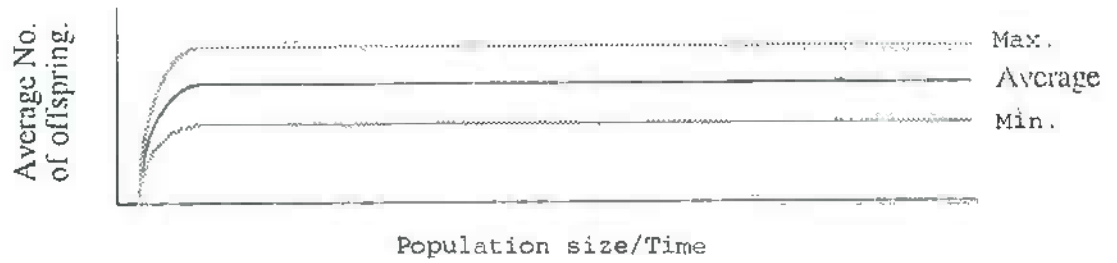
A common character of all these species is that they are comparatively long lived, feed throughout adulthood and colonise hosts which once found/conditioned, can potentially support many individuals. I believe that it is these characteristics which have facilitated

the evolution of aggregation pheromones and ultimately explain why, if their function is primarily sexual, con-specifics of the same sex as well as potential mates are attracted. The resulting aggregation will then increase the potential for sexual selection. Combined with a relatively long adult life span it is perfectly feasible that such selection pressures may have shaped some of the details of aggregation pheromone systems.

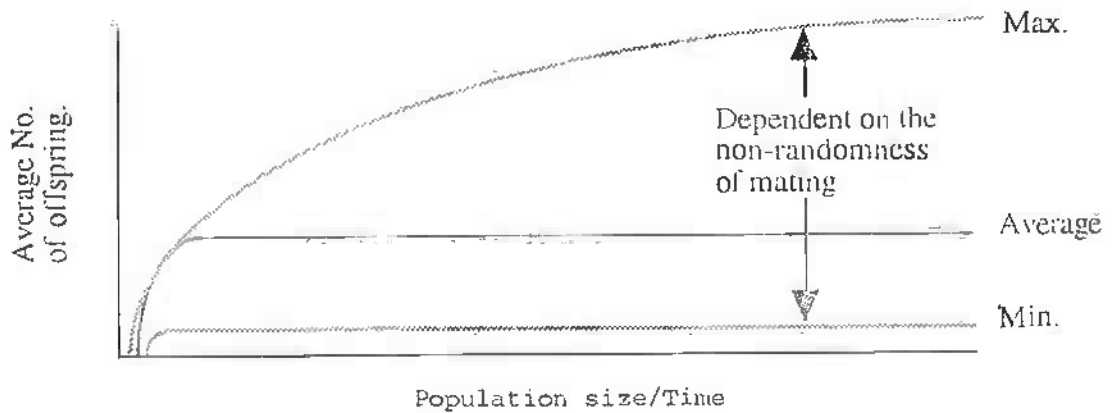
To sum up, both sexual and collective "conditioning" of the host functions of aggregation pheromones are possible given the evidence currently available. It is likely that especially in those cases where a conditioning function is implied, that both kinds of benefits are occurring for the signaller. I have looked at evidence for pheromone function from the patterns that can be recorded now, however, it must be remembered that such pheromones may have initially evolved in a very different context and may have had a different function. For example, *Prostephanus truncatus* is currently found as a pest of stored maize and Cassava, but is also known to be a wood borer (Hodges 1994), a habit which may have been influential in determining its use of pheromones. As Schlyter 1989 says, "...a good analysis of the functioning of the present system is no substitute for an analysis of the origin of the system. Here I have put forward just a few possible ideas about the adaptive function of aggregation pheromones. This is not an exhaustive list of possibilities by any means. My real aim is to get away from the need to use group selectionist explanations to explain this phenomenon that happens to result in group forming.

Fig. 7: Model to show how the average number and survivorship of offspring might vary for females on host types 1 and 2 as an aggregation of conspecifics forms on the host

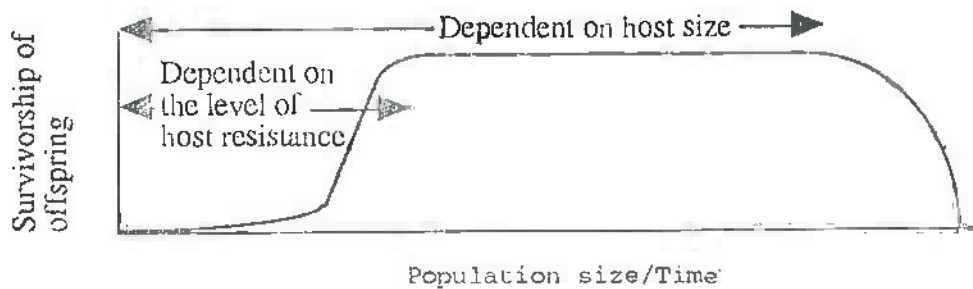
7a: Model to show mean and variance of offspring per female as an aggregation forms.



7b: Model to show mean and variance of offspring per male as an aggregation forms.



7c: Model to show how survivorship of offspring varies as an aggregation forms on host type 1.



7d: Model to show how survivorship of offspring varies as an aggregation forms on host type 2.

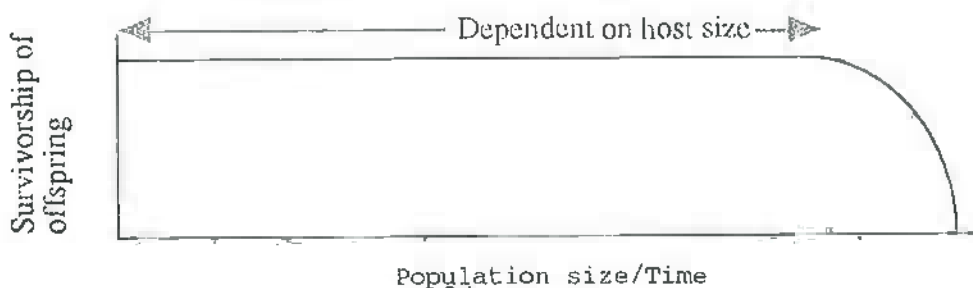
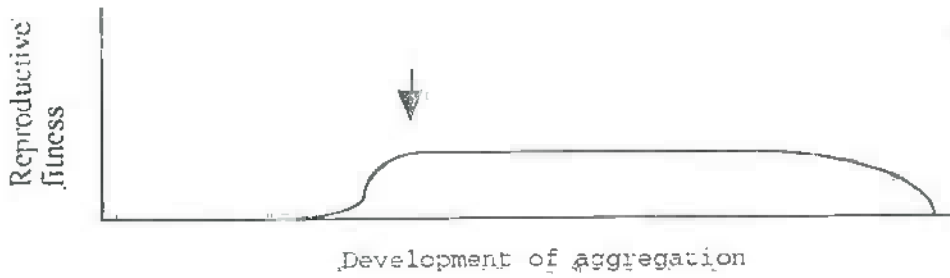


Fig. 8: Model to show how reproductive fitness varies for either males or females an aggregation of conspecifics forms on a host.

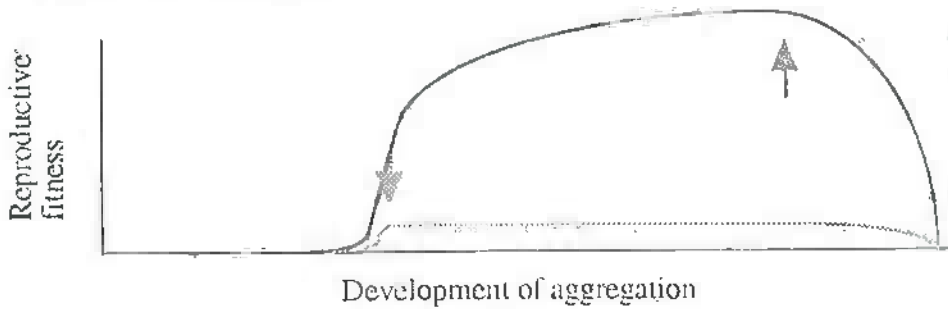
8a: Females on host type 1.



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↓ All arrows indicate the point where maximum reproductive fitness is attained and no further benefit could be gained by attracting more mating partners.

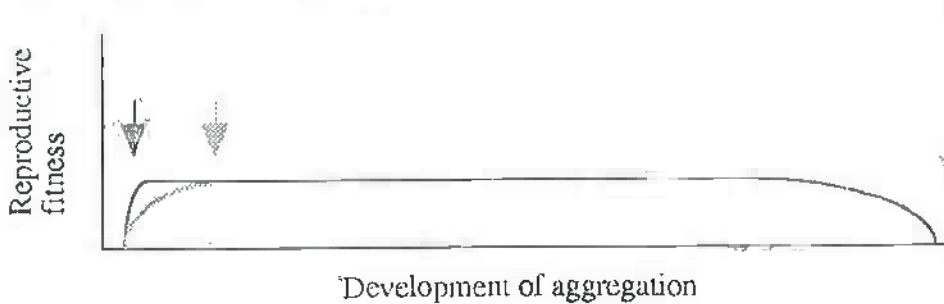
8b: Males on host type 1.



↑ Males of high competitive ability

↓ Males of low competitive ability

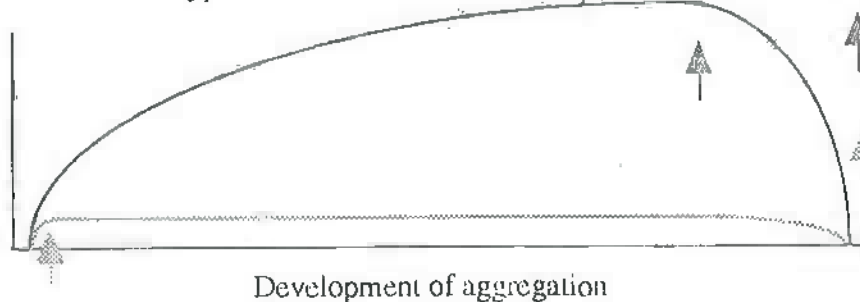
8c: Females on host type 2.



↓ Where females do not gain much reproductive fitness from additional mates

↓ Where females only gain maximum fitness when many males are available to "choose" between or to contest between themselves.

8d: Males on host type 2.



↑ Males of high competitive ability

↓ Males of low competitive ability

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