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An empirical study of the evolutionary significance of cannibalism in tree-hole mosquitoes (Diptera : Culicidae) from ecological and biological perspectives

by
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Summary

To be adaptive, optimality theory suggests that behavioural traits should maximise the fitness of the carrier by spread of genotypes through a population. Expression of these traits are expected to vary with biological and ecological factors modifying fitness consequences. This study investigates the consequences of such factors to the dynamics of cannibalism in aquatic larvae of three tropical tree-hole mosquitoes, *Aedes aegypti*, *Trichoprosopon digitatum* and *Toxorhynchites moctezuma* and examines the adaptive nature of cannibalism in the context of these findings.

1. Expression and rate of cannibalism varied with food and density levels in *A. aegypti*. When food limits development, a greater percentage cannibalise and at a higher rate. At high larval density, a higher proportion cannibalise, but at a comparatively lower rate, probably due to physical interference of feeding. No nutritional benefits of cannibalism were reflected in adult fitness parameters of size and development time.
2. No fitness consequences, in terms of adult size and development time, were detected at intermediate food levels, between *T. digitatum* larvae allowed and larvae prevented from cannibalising, despite recurrent cannibalism.
3. Comparison among clutches of *T. digitatum* revealed a significant variation in propensity to cannibalise.
4. Inter-species comparison of cannibalism in kin and non-kin situations revealed no evidence kin selection, despite disparity in expected benefits amongst species and seemingly obvious fitness advantage to *T. moctezuma*.
5. The adaptive significance of cannibalism is considered in the light of these discoveries. Anomalies can be explained by viewing cannibalism as a strategy, representing a trade-off between relative costs and benefits in particular ecological and biological conditions, and conflict with other behavioural strategies such as oviposition and predator avoidance, which maximises reproductive success. Focused empirical studies provide a powerful tool for identifying anomalies and generating refutable trade-off hypotheses.
6. Population and community level consequences of cannibalism are examined together with their probable epidemiological implications.

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Chapter 1 Introduction

Cannibalism, defined as intraspecific predation, the act of killing and consumption of conspecifics, is a taxonomically widespread phenomenon which would appear to be antithetical to the goal of maximising reproductive success, (axiomatic to adaptive behavioural studies). However, cannibalism has been shown to have diverse fitness implications, (see Fox, 1975, Polis, 1981, Elgar & Crespi, 1992 for reviews) acting on an individual level, for short-term behavioural decisions, (such as diet and patch choice), on an evolutionary scale, with respect to social, territorial and courtship behaviour and recognition of kin and on a community level, with respect to population dynamics (Cushing, 1991 & Polis, 1981) and the persistence and coexistence of species (Dong & Polis, 1992). Can the occurrence and expression of the cannibalistic trait be explained adaptively?

A convincing role of adaptation, defined here as "a phenotypic variant that results in the highest fitness among a specified set of variants in a given environment" (Reeves & Sherman, 1993), is perceived if natural variation in trait expression is correlated with ecological, morphological, physiological or behavioural variables that modify costs and benefits in terms of reproductive success. Therefore, to determine if cannibalism is adaptive, perceived costs and benefits of eating conspecifics and any factors modifying fitness consequences need to be identified.

The main, most frequently cited, costs and benefits of cannibalistic behaviour are identified in table 1, together with references to supporting literature.

Table 1; Costs and benefits of cannibalism

Adaptive siginificance - benefits	**Examples**	**References**
Nutritional	Mantids, Hemiptera, Snails - Enhanced survival, growth and fecundity in limiting conditions. Sticklebacks, Ladybirds - Source of essential nutrients *Uthesia* caterpillars - Transfer of protective toxins	Fagan & Hurd, (1991), Parajulee & Philips, (1995), Baur, (1994) Bellesisles & Fitzgerald, (1993), Agarwala & Dixon, 1992) Bogner & Eisner,(1992)

Reduce intraspecific competition	Damselfly, mice, pike - reduces detrimental fitness consequences of high density (scramble, physical and chemical inhibition)	Fincke,(1994), Southwick, (1955), Kipling & Frost (1970) & Fischer, (1960), quoted in Fox, (1975).
Sexual selection, killing to gain reproductive advantage	Primates and Lions - males kill competitors offspring and mate with females Males eat competitors	Hamai et al., (1992), Betram, (1975) but see Mestel, (1995) for inadequacy of evidence see Polis, (1981)
Parental manipulation	Corixidae, social insects - Parents recoup investment when food is scarce. Brood reduction in raptors Female provides trophic eggs	Pajunen & Pajunen, (1991), Wilson, (1971) O'Connor, (1978), Stinson, (1979) see Polis, (1981)
Adaptive significance - costs		
Risk of retaliation	Alligators, fish, scorpions - size-dependent asymmetry	Rootes & Chabreck, (1993), Thibault, (1974), Kipling, (1983), Polis, (1980),
Species-specific disease and parasite transmission	Tiger salamanders - expression of cannibalism correlated to bacteria levels	Ladle, (1991)
Loss of sociality benefits by reducing group sizes	Gregarious Willow Leaf beetle - addition of larvae increases survival	Breden & Wade, (1989)
Cost to inclusive fitness by eating relatives	*Labidomera clivicollis*, development must be speeded up by an extra 2 days to compensate for the cost to inclusive fitness of eating siblings,	Eickwort, (1972).

Many ecological and biological factors have been found to modify the relative importance of these costs and benefits and so warrant consideration in any explanation of the distribution and dynamics of cannibalism. Ecological examples include the availability of alternative food. Where alternative food sources are abundant, magnitude of nutritional benefits of cannibalism are reduced because growth, development and survival rates are already high. Cannibalism rates in the aquatic isopod, *Saduria entomon,* were found to depend on the depth of water, in deep zones, alternative food was scarce and cannibalism considerably higher, (Leonardsson, 1991). Similarly, variation in rates of cannibalism between geographic populations of salamander larvae in Israel were found to be correlated to abundance of other food sources (Degani, G, 1993) and in kestrels, the abundance of small mammal prey was inversely related to frequency of cannibalism, (Boutolotti *et al.*, 1991).

With time and perceived temporal permanency of a habitat, the occurrence and rates of cannibalism often vary, with higher rates at the end of a season or in very ephemeral habitats, when time is a limiting factor and hence fast development (and so food supply) at a premium. Incidence of cannibalism in rock pool corixids was found to increase as summer progressed, (Pajunen & Pajunen, 1991) and the expression of a cannibalistic morph by teleosts of the genus *Poeciliopsis* is associated with ephemerality of habitat, (Thibault, 1974).

Other studies have revealed a sex difference in propensity to cannibalise (see Pajunen & Pajunen, 1991 for Corixids, Caracamo & Spence, 1994, for water striders, Tarpley *et al.*, 1993, for the South Western Corn-Borer, Mertz & Carthon, 1973 for *Tribolium* and Church & Sherratt, submitted, for the mosquito *Trichoprosopon digitatum*) which may be equated to differential investment and energy requirements in the sexes, e.g. the additional costs of oogenesis and host location for blood feeding (see Sota & Mogi, 1994 for examples of cost) for female mosquitoes.

Absence of cannibalism can be explained by biological constraints, infrequency of cannibalism in birds has been attributed to rapid and determinate growth of fledglings which, combined with swallowing prey whole, makes young/sibs too large to consume. Significantly, cannibalism is mainly confined to falconiformes and strigiformes which tear food (Bortolotti *et al.*, 1992). With reference to mosquitoes, two main methods of larval feeding have been identified., a la carte (nibbling) and table d'hôte (sweeping hair like brushes and creating a feeding current) (Marshall, 1938). It can be envisaged that mode of feeding employed can similarly directly control ability to cannibalise.

It may be expected that cost of cannibalising will increase with degree of relatedness between cannibal and prey. Hamilton (1964) coined the term inclusive fitness as an individual level representation of genotype frequency, showing that a gene can spread through a population through its' effects on reproductive success of animals other than direct offspring. He used this theory to calculate the conditions under which a gene might spread through and be sustained in a population, namely if benefit (b) to recipient exceeds cost (c) to the donor, where benefit is devalued by the coefficient of relatedness, denoted "r", (the probability of sharing rare genes) i.e. where $rb > c$. Although co-operation can occur between unrelated individuals, namely when b is large and c small and help likely to be recompensed (for example blood-meal sharing in vampire bats, (Wilkinson, 1964)), clearly, reciprocal altruism (complementary acts in the interest of others but at a cost to oneself) is more likely to benefit fitness and occur between relatives as kin groups consequent a viscosity of the gene pool, whereby relatives are more likely to share the " helping" or, in this case "avoid cannibalising" gene.

Although kin selection theory predicts that, in terms of inclusive fitness, cannibalistic behaviour is more likely to spread and be maintained if kin can be recognised and avoided (especially where oviposition is aggregative and recognising only neighbours as kin will be insufficient), benefits to inclusive fitness of recipient and donor, for a given r-value, can be expected to vary according to frequency of contact, (dictating the number of costs/benefits that can occur), cost of recognising kin (see Waldman, 1987, Waldman *et al.*, 1988, for mechanisms of kin recognition) and inequality of the pay-offs shown below;

		Recipient	
		gain	loss
Donor	gain	co-operation	selfishness
	loss	altruism	spitefulness

Abundant evidence for kin discrimination in cannibalistic decisions exists (see reviews in Fletcher & Michnev, 1987 for eusocial and semi-social insects, Eickwort, 1973, Agarwala & Dixon, 1993 for ladybirds, Nummelin, 1989 for water striders and Waldman ,1987).

Several studies have indicated a heritable, genetic basis to variation in cannibalistic tendencies, for example between populations of the South-western Corn-Borer and fish of the genus *Poeciliopsis*, where hybrids displayed intermediate levels of cannibalism (Tarpley *et al.*, 1993, Thibault, 1974), between laboratory strains of Flour beetles (Stevens, 1989) and mites (Croft, & McMurty, 1972). and through differences in cannibalistic propensities between relatives, sexes and ages (see Fox, 1975, p 96).

Both a genetic basis and an adaptive explanation for cannibalism are supported in the literature, according to the previously mentioned adaptive criterion of dynamic trait expression correlated to changing levels of fitness governing factors. For cannibalism, such factors include food levels, density, age, relatedness, size and hunger, which consequent fluctuations even between daily sampling periods, (examples include rotifers, (Hurlbert *et al.*, 1972, Notonectids, (Fox, 1975b) and yellow perch, (Forney, 1974) quoted in Fox, 1975).

Study Aims

Although cannibalism can occur among and within all life-stages through infanticide, gerontophagy, oophagy, and can take place, for instance, during competitive interactions or during mating and courtship, due to time constraints, this study will focus on cannibalistic interactions within immatures. The adaptive nature of cannibalism within the aquatic, larval stages of three species of tropical, tree-hole mosquitoes (Diptera : Culicidae), will be explored, with two main objectives;

1. **To determine if food supply, density, relatedness and heritable genetic predisposition explain variability in expression of the cannibalistic trait.**

2. **To determine, in a sub-set of conditions, the nutritional advantages that cannibalism confers to adult reproductive success.**

Chapter 2 Materials and Methods;

2.1 Biology of the study species

Mosquitoes fall into the nematoceran family, the Culicidae, of the Dipteran order of insects. This family is divided into three sub-families, the Anophelinae, the Culicinae and the Toxorhynchitinae of which only the former two groups are blood-feeders. Species used in this study belong to the latter two subfamilies, *Trichoprosopon digitatum* (Rondani), *Culex* (*Culex*) *mollis* and *Aedes aegypti* (Linneaus) of the Culicidae and *Toxorhynchites moctezuma* (Theobald) of the Toxorhynchitinae. Mosquitoes are not only a convenient species to use, being abundant, diverse, easy to rear and have some documented cannibalistic tendencies (referred to in this text), but also introduce an applied aspect to the study of their behaviour. They not only represent a public nuisance but also, more seriously, are vectors for many widespread, potentially lethal diseases for both humans and their livestock: malaria, yellow fever, dengue fever, encephalitis and filiariasis being commonly cited examples. As cannibalism can be a key mortality factor (for example contributing 85 % mortality in *Pardosa lugubris* spiderlings, (Edgar, 1962, 1971)and 63.7% mortality in alligators of age 11 months or over, Rootes & Chabreck, 1993, causing two-year cycles of age-structure in dragonfly nymphs, Vanbuskirk, 1992) and is known to affect population dynamics (both size and temporal stability) and persistence, it bears consequences for the control of such species.

The cosmotropical species, *A. aegypti* L. is of medical importance as it is the main vector of Yellow Fever and Dengue Fever. In common with other species of its genus, eggs are laid singly, above the water level of small receptacles. It is mainly urban in its distribution, as humans are a main blood host, ovipositing into artificial containers such as water tanks, tires and bricks. The females have been documented to oviposit into a number of different sites, depositing a number of eggs into each receptacle (Apostol *et al.*, 1993), with an average of approximately 93 eggs per oviposition and four days between ovipositions (Seawright, Dane & Weidhaas, 1977). The desiccation-tolerant eggs diapause until water levels rise, or until water levels have changed several times. This may help to insure that larvae hatch into larger, more permanent water bodies. A method of instalment hatching has been widely documented in this genus (Gillett, 1955, 1955b, Gillett *et al.*, 1977, Livdahl *et al.*, 1984, Edgerly *et al.* 1993) whereby all eggs do not hatch at the same time. High densities of large larvae suppress hatching as does absence of larvae. Hatching rate is maximised by intermediate densities of small larvae or low densities of large larvae.

This response is thought to be mediated through levels of dissolved oxygen. As bacteria accumulate in the water and on the surface of eggs, oxygen levels drop. As larval density increases, so bacteria (food) levels drop due to grazing (and possibly larval respiration) and hatching is suppressed by the consequential increase in dissolved oxygen. This mechanism, together with variation in hatching stimuli within a clutch, has been proposed as a method to assess and avoid conditions of overcrowding and limited food supplies that may promote cannibalism, (Livdahl, 1982, Koenekoop & Livdahl, 1986).

Although the larvae are mainly detritivores in all instars, cannibalism has been documented in some but not other species of *Aedes* especially in low food conditions, for example in *A. triseriatus* (Livdahl, 1982, Koenekoop & Livdahl, 1986) and briefly in *A. aegypti* (MacGregor, 1915) and *A. cantans*, (Renshaw, *et al.*, 1993) but not in *A. sierrensis* (Broadie & Bradshaw, 1991). In all reported cases cannibalistic events were highly size-dependent. Koenekoop & Livdahl found that in *A. triseriatus* cannibalism of first instars by 4th instars reduced from 30% at 1 hour old to less than 10% at > 24 hours old probably due to increased agility of first instars.

T. digitatum is an egg raft-laying mosquito which breeds in natural and man-made water receptacles. The females produce usually one raft of 60-80 eggs (Aitken *et al.* 1968) and can not withhold eggs, (O'Malley, 1986). The females then guard the eggraft on the water surface between the mesothoracic legs for 24 to 30 hours, probably to reduce the risk of displacement during heavy rainfall (Lounibos & Machado-Allison, 1987). An incidental consequence of this is that it insures that clutches are kept discrete. However due to the aggregative nature of *T. digitatum* oviposition (Sherratt & Church, 1994) an extra precaution was taken to isolate pure clutches during experimentation, by rejecting irregularly shaped rafts. The eggs are incapable of diapause and all hatch at roughly the same time. All four larval instars feed predominantly on micro-organisms and detritus (Seifert & Barrera, 1981). Larger larvae have been documented to be able to survive food shortages (Busck, 1908, cited in Zavortink *et al.* 1983) and desiccation (Galindo *et al.*, 1951). Cannibalism has been noted to be a common feature but usually confined to size-dependent interactions with larger instars eating smaller ones, (Seifert & Barrera, 1981, Sherratt & Church, 1994).

C. mollis is another egg-raft laying mosquito but, in contrast to *T. digitatum*, the females desert the egg rafts (which tend to stick to receptacle sides) immediately. Egg rafts vary in shape but generally are more longitudinal than circular. It is not presently known whether this species is capable of laying a second raft. The larvae once again are detritivores and little is known of cannibalistic tendencies although some findings indicate absence of cannibalism (Seifert & Barrera, 1981).

T. moctezuma, (see figure 2.1) belonging to the only genus of the culicid subfamily Toxorhynchitinae, also lays eggs in both natural and man-made water receptacles. However it lays single eggs, dropping them from the air onto the water surface. Females can oviposit suddenly and disperse eggs between a number of sites due to continuous production of mature follicles and an asynchronous pattern of oogenesis, (Steffan & Evenhuis, 1981). Although some contention exists, (see Chadee, Hubbard & Corbet, 1987) females generally deposit several eggs, up to as many as 84 (Trpis, 1981), before leaving a receptacle, (Steffan & Evenhuis & Personal observation). A time interval of between one day -one week separates ovipositions, (Trpis, 1981). Eggs hatch after 40-60 hours. The first instars can then last a four-six day starvation. Fourth instars, by prolonging pupation and reducing metabolic rates, are very tolerant of starvation. The larvae are voracious predators of other active invertebrates (including other mosquito larvae) and highly cannibalistic, feeding even on larger conspecifics and pupae. Prior to pupation, an act of compulsive killing of surrounding invertebrates commonly occurs in some but not all cases, possibly to reduce the risk of predation during the pupal stage (Corbet & Griffiths, 1963). The predatory behaviour of larvae is that of a sit and wait ambusher. Where potential prey are approached head-on, attack is generally avoided, (Breland, 1949, Muspratt, 1951).

Figure 2.1; Adult male T. moctezuma

2.2 Description of Study Sites

Experimentation was split into two parts: laboratory work on *A. aegypti* during May and June and field-based study in secondary seasonal deciduous forest surrounding the Simla Research Station, Arima valley, Trinidad (10°42'N, 61°17'W) (see figure 2.2) in July, 1995. Investigation for roles of kin selection and genetic, heritable components to cannibalism were completed on natural populations, as a quantifiable variation in relatedness, unavailable in highly inbred laboratory strains, was required.

Figure 2.2; Example of secondary seasonal forest:

Trinidad covers an area of about 450 km² and marks the Southern-most extent of the West Indies, situated 15 km north of the coast of Venezuela, from which it is separated by the Gulf of Paria.

Arima Valley is situated at the foothills of the Northern Range Mountains towards the North of the Island, (see figures 2.3 and 2.4). Much of the forest surrounding Arima is secondary due to previous utilisation as commercial plantations. Commercial species (both native and exotic) such as cacao, citrus, plantain species, bamboo and mahogany, *Swietenia* spp. and *Lecythis zapucajo* are still widely evident, together with recolonising forest species. The climate, due to equatorial low pressure movements, is seasonal with a marked wet season of monsoonal rains running from May to December and a dry season extending from January to April. Temperature remains fairly stable throughout, with the mean monthly maximum varying from 30.2 to 32.1°C and the minimum from 19.8 to 22.1°C. (Walter & Leith, 1967).

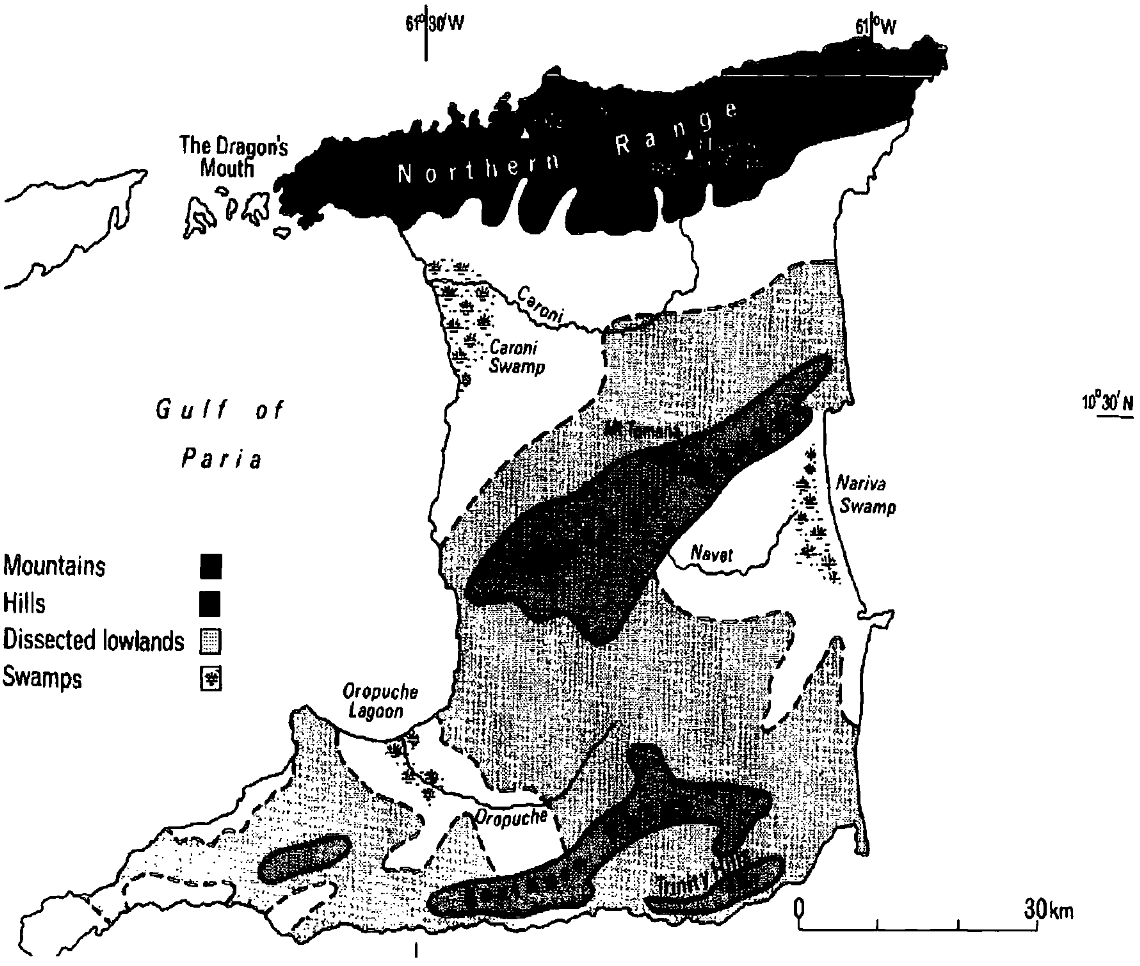


Figure 2.3; Relief map of Trinidad:
Adapted from: *The West Indies* (Evans, 1973, p43).

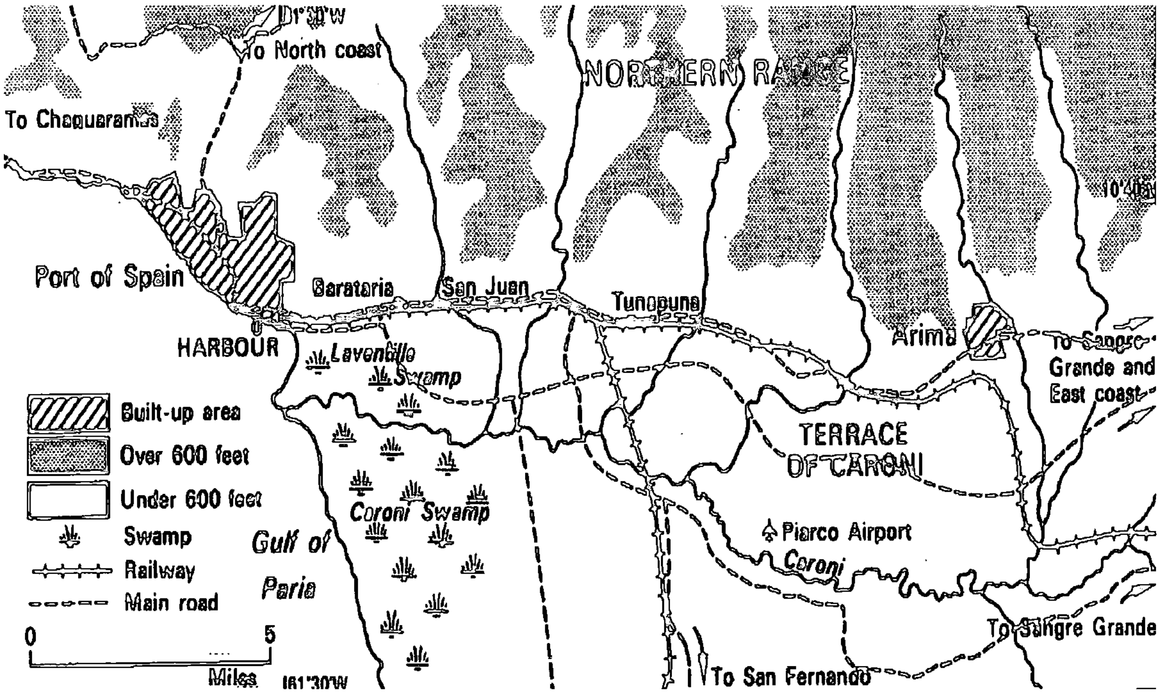


Figure 2.4; Detailed map of Arima and surrounding area:
Adapted from: *A modern secondary geography of the West Indies* (Bent, 1971, p174).

The generalised life-cycle of mosquitoes is depicted in figure 2.5. As the larval stage of mosquitoes is aquatic, taking in air through a respiratory siphon from plant roots or, more commonly from the water surface, they are generally more common in the wet season. Therefore collections were carried out throughout July, 1995. Oviposition occurs either directly into or onto the edge of water bodies. All species used in this study are known as tree-hole mosquitoes, meaning that adults lay in small water bodies (including both natural and artificial containers, for example cacao husks, *Helliconia* bracts, bamboo joints, leaf axils of bromeliads, calabash, tyres and paint pots, see Lounibos & Machado-Allison, 1983) as opposed to larger, more permanent ponds and lakes.

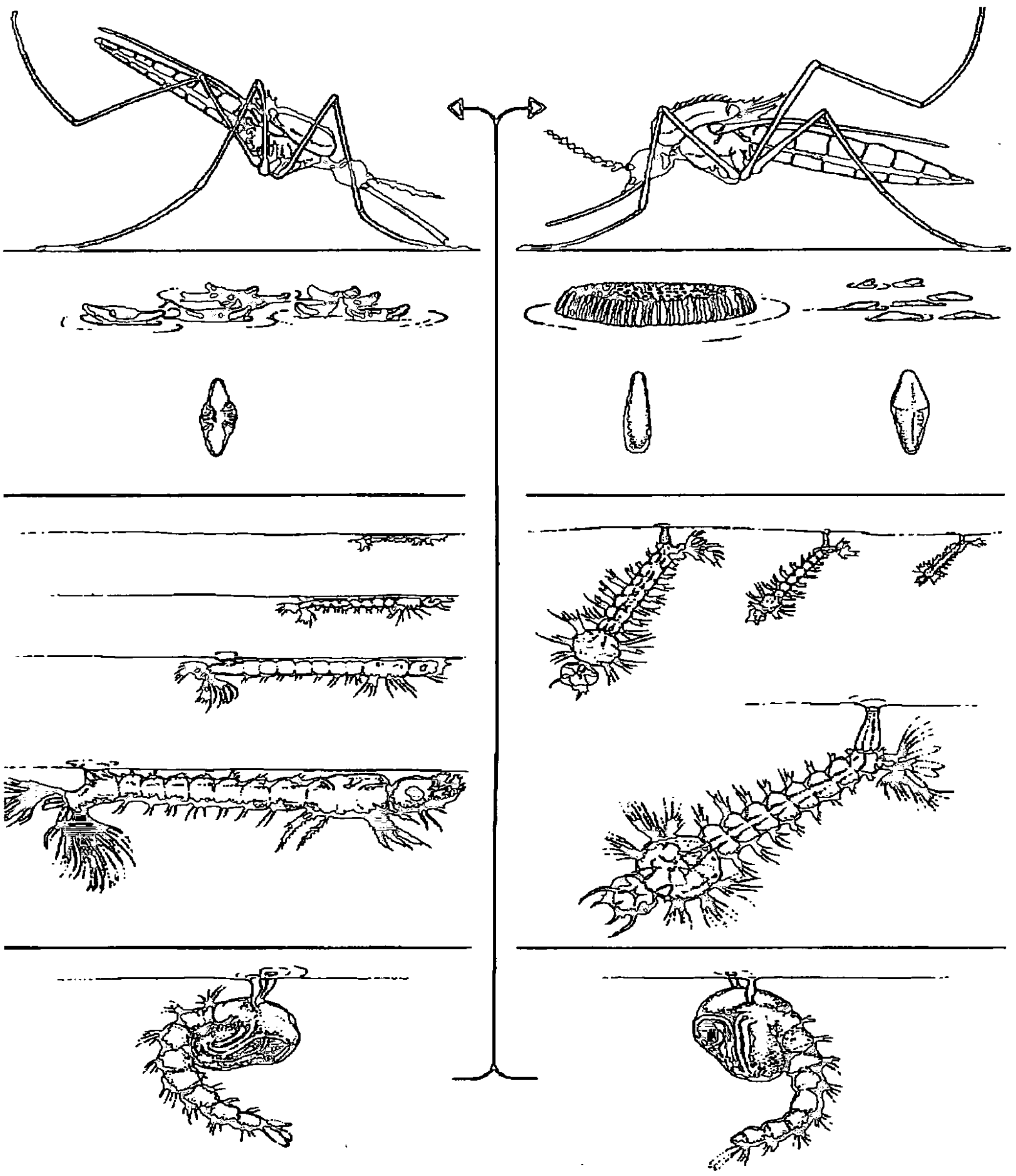


Figure 2.5; Generalised life-cycle of the mosquito:
Adapted from: *A colour atlas of medical entomology*. Burgess & Cowan (1993, p30).

Mosquito eggs were collected from two main sources, 500 ml white plastic beakers and nut pots, fallen from the tree, *Lecythis zapricajo* (Zabucajo) (see figure 2.6). The pot is the woody endocarp of the seed which are discarded from the tree after an operculum breaks off, releasing the seed. These were increasingly relied upon as they not only proffered protection from rain and sun but also proved attractive as the inner lining decays into the water, forming a thick, dark organic liquid. Egg rafts were collected at 06:30 and 17:30, to coincide with peak oviposition of the raft -laying species whilst *Toxorhynchites* eggs were collected every 1-1.5 hours from 13:00 - 16:00 to coincide with its peak in oviposition (see O'Malley, 1986 for oviposition periodicity). This helps to insure that larvae used in experiments were of uniform age and from the same clutches.

Figure 2.6; A typical nut pot of the tree, *Lecythis zapucajo* (Zabucajo)

2.3 Experimental protocols;

Experiment 1 : Effect of density and food supply on the fitness consequences and occurrence of cannibalism in the yellow fever mosquito, *Aedes aegypti*
Experiment 2 : Evidence for a genetic component to the propensity to cannibalise and examination of possible nutritionally-derived fitness consequences of cannibalism at intermediate food levels in field populations of *Trichoprosopon digitatum*.
Experiment 3 : Cross-species investigation for evidence of kin discrimination in cannibalism.

In all cases larvae used as replicas in experiments were of equal age and kept in the same conditions of food, light and temperature, as hunger has been documented to effect level of activity and hence riskiness of behaviour, feeding rates and prey choice thresholds, (Johansson, 1993, Fagan & Hurd, 1991, Juliano et al, 1993), light to effect activity (Macrae & Croft, 1993) and age and life history stage to effect rates of cannibalism (in the corn-borer cannibalism was highest in 15-18 day-olds, (Tarpley *et al.* ,1993), pupal weight of *T. splendens* depends only on food consumed in the fourth instar (Jones, 1993).

2.3.1 Experiment 1 ;

This experiment aims firstly to determine if the mosquito, *A. aegypti* cannibalises and secondly, if they do, to determine under what ecological conditions cannibalism is beneficial. Two main conditions were varied, density and food levels.

Eggs were obtained from the Liverpool and London Schools of Tropical Medicine and kept in a desiccating chamber until hatching. Eggs were hatched in 26 ^{0}c water and larvae were supported on a nutrient broth (24g desiccated liver powder, 16g yeast ground up and 1 litre of distilled water). All eggs and larvae were kept in a 26 ^{0}c constant temperature room. The ability to tolerate desiccation was used to create the necessary size difference. Larvae hatched on the 23/05 were used as potential cannibals on the 26/05. Newly hatched first instars were used as prey. Experiments were set up in ice-cube trays (see figure 2.7), each cell being independent, with the following components;

1. Normal (x 15) - 1 Large larvae, 19 ml water, nutrient broth and 3 first instars.
2. Low Food (x 10) - 1 large larvae, 19 ml water, 3 first instars.
3. High density (x 10) - 3 large larvae, 19 ml water, nutrient broth and 3 first instars.
4. Control (x 10) - 3 first instars and 19 ml water. To quantify non-cannibalistic mortality

Every 24 hours the number of missing first instars were recorded and the remaining replaced with three more, newly hatched first instars. This was repeated each day until pupation. On emergence, the sex and size of adults was recorded. The length of the hind tibia was used as a reliable measure of adult size (see Godfray, 1994)

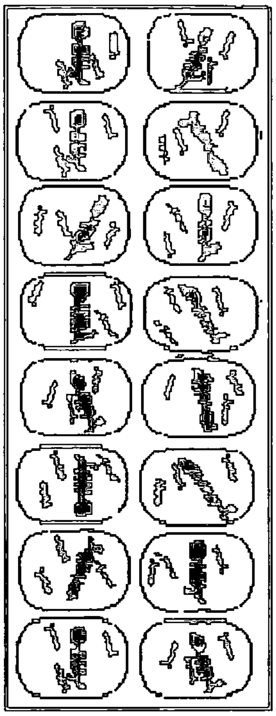


Figure 2.7; Graphic illustration of a "normal" condition ice-cube experiment

2.3.2 Experiment 2 ;

This experiment aims to provide evidence for fitness consequences of cannibalism and to discover if any variability in trait expression can be explained genetically.

Survivorship, size and development time have been selected as indicators of reproductive success. Evidence for reproductive benefits of large size in females are clear, including evidence for greater longevity (Hu *et al.*, 1993, in *Annopheles*), larger flight range giving greater probability of host and oviposition site location (Lounibos, 1994, in Annopheles) and greater fecundity, for example in the form of higher egg numbers per raft in *T. digitatum* (Church & Sherratt, in press) and in *Aedes sierrensis* (Hard & Bradshaw, 1993) (see also Service, 1977 & Renshaw *et al.*, 1994, for *A. cantans*, Colless & Chellapah, 1960 for *A. aegypti*, Corbet & Griffiths, 1963, for *Toxorhynchites*). However for males, benefits are more contentious. Yuval *et al.* ,1994, found no difference in feeding or swarming ability of male *Annopheles* with increasing body size however Benjamin & Bradshaw, 1994, report increased probability of reproductive failure with decreasing size in *Wyeomyia smithii*. It may be that larger males gain an advantage through ability to fly at lower temperatures, (Hard

& Bradshaw, 1993, in *A. sierrensis*). Development time can increase reproductive success by reducing generation time.

Previous study of *T. digitatum* cannibalism at high and low food levels suggested a benefit to cannibalism advanced through enhanced longevity in low food conditions. However a direct reproductive benefit through increased adult size or reduced development time, was not found at high food conditions and was not available for low food conditions due to failure to pupate, (Church & Sherratt, 1995 and experiment 1 above in *A. aegypti*). Investigation of cannibalism in *A. triseriatus* suggests that it is only at intermediate food conditions that cannibalism provides a reproductive advantage in terms of adult size (Livdahl, 1982). This may be because at high food levels food is not a limiting factor to larval development, and in low food conditions, opportunity to cannibalise can not compensate nutritionally enough to allow pupation. In low food, competition for limited food that the smaller conspecifics cause, could outweigh nutritional benefits gained from eating first instars. A similar concept has been used to explain why, as density of small conspecifics increase, cannibal size decreases in damselfly larvae, (Anholt, 1994). Therefore food conditions intermediate to those applied previously of 19 ml spring water (low food) and 19 ml 3g/l mixture of dried, white bread crumbs in water (high food), Church & Sherratt (submitted) have been used.

By using strains of inbred mosquitoes of unknown genetic relatedness, the role of a heritable component to the propensity to cannibalise and kin discrimination on rates of cannibalism, can not be assessed. Besides explaining variability in cannibalism, if significant, these factors, unless controlled for, can act to confound effects of other factors in laboratory study of cannibalism. Inter-clutch variation in cannibalistic tendencies will be determined from field populations of *T. digitatum*.

Sixteen three-day-old individuals were randomly chosen from each of 16 regularly shaped egg rafts of *T. digitatum,* reared in plastic cups containing 200 ml of a 3g/litre mixture of dried bread crumbs in water. One individual was placed in a cell of each of 16 ice-cube trays. Each ice-cube tray therefore held one individual from all 16 clutches. Larvae were kept in food conditions of 19 ml water with three, temporally-spaced, standardised pinches of bread crumbs. All trays were covered to reduce light levels to more natural levels and were kept at ambient temperature (20-30 ^{0}c). To prevent larvae migration between cells, a thin layer of boot wax was applied above the water line to each cell. Eight of these trays were given the opportunity to cannibalise in the form of three recently emerged first instars (+ reps). Each day, trays received first instars from the same clutch to control for inter-clutch differences in cannibalism avoidance. The number of first instars absent after each 24 hour period was recorded and all first instars replaced daily until pupation. Sex of adults on emergence was also recorded. Adult size was measured by length of hind tibia.

2.3.3 Experiment 3 ;

Tree-hole mosquito larvae all live in confined water bodies with no chance of dispersal, in accordance with the first precept of reciprocal altruism. However, although altruism is more likely between relatives, the biology of oviposition and egg hatching and differing propensity to cannibalise between species affects the frequency of possible kin cannibalism contact and hence the advantages of kin selection. As *T. digitatum* and *C. mollis* eggs hatch simultaneously and cannibalism in the former is known to be highly size-dependent, situations where kin have the opportunity to cannibalise are not likely to occur. However in the case of *T. moctezuma*, likelihood of kin cannibalistic meetings are significantly greater as, although eggs hatch simultaneously, cannibalism frequently occurs within the same age and size classes. With respect to inclusive fitness it would be expected that kin discrimination would be more likely in the latter species. Hence the following outcomes are hypothesised;

- **H1 50:50 consumption of kin and non-kin prey by *T digitatum* and *C. mollis***
- **H2 Preference for non-kin conspecific prey in *T. moctezuma***

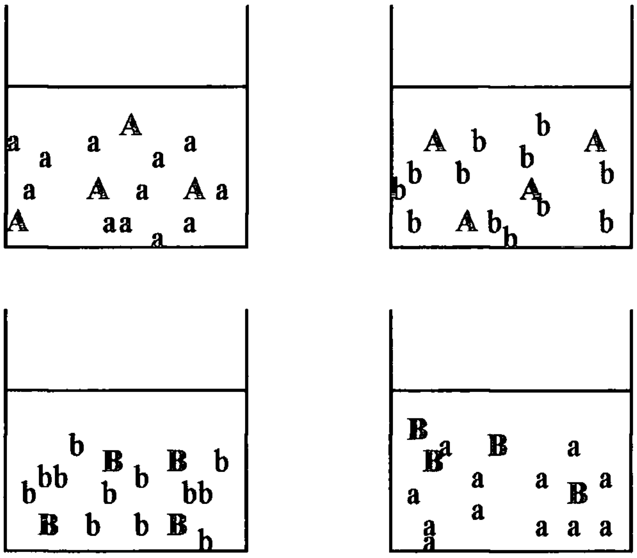
This proposed importance of kin contact to the evolution of kin discrimination during cannibalism was eloquently substantiated by Wades' work on *Tribolium confusum*. When larvae were placed in the presence of sibling eggs, kin discrimination built up in only three generations, with attack rate being lowest on full sibs, (Wade, 1980).

This experiment comprises a cross species investigation (using the species mentioned above) to test for kin selection by comparing dynamics of cannibalism in kin and non-kin situations. Due to the varying tendencies to cannibalise, different experimental designs were used.

Experimental designs;

2.3.3.1

As cannibalism is largely size-dependent and eggs hatch simultaneously, size differences had to be created "artificially" in *T. digitatum* and *C. mollis*. In total, three sets of 10 clutches were set up on consecutive mornings using eggs collected from the previous morning. On hatching, clutches were split into high and low food conditions. In the high food condition 15 larvae were placed in 100 ml of 6g/litre dried, white grated bread crumbs in water. Approximately 50 first instars were placed in 100 ml 1g/litre bread crumbs in water, to create "low food" conditions. After three days (replacing the high food conditions to avoid suffocation) the high and low food treatments for each clutch were randomly assigned to two groups. Two groups of 4 "large", high food and 2 groups of 12 small larvae were randomly selected. Clutches were arranged in pairs with a group of small larvae of clutch A and clutch B being assigned to the two large larvae groups of clutch A and the same for large larvae from clutch B (see figure 2.8). A pairwise design was chosen to account for the confounding effect that any inter-clutch variation in cannibalism (either due to genetic variability in tendencies or size) would have. Experiments were set up in plastic beakers with 50 ml tap water and low food conditions. The number of small larvae absent was recorded every 24 hours until all four large larvae reached pupation or death. The sex of the emerging adults was recorded.



A and B represent separate clutches, capitals depict cannibals and lower case, prey.

Figure 2.8; Experiment 3; Graphical illustration of the pairwise experimental design employed for *T. digitatum* and *C. mollis*

2.3.3.2

For *T. moctezuma*, this design would have been inappropriate as cannibals could not be identified due to cannibalism between the same size classes. Therefore a design with groups of four larvae, two from each of two clutches was adopted. As a precaution against the potentially scattered nature of oviposition, eggs were collected from nut pots frequently (every 1-1.5 hours over peak oviposition time) and only clutches of $\leq$ 10 eggs were used, to attempt to isolate genuine clutches. All larvae of each clutch were measured from head to the base of the siphon under the microscope so that similar sized individuals were chosen and hence size bias minimised. To identify kin each larvae was painted with a randomly chosen spot of paint on its thorax. To insure that the paint procured no handicap, larvae were left overnight, with the same quantity of food (larvae of *T. digitatum* and *C. mollis*) to maintain standardised satiety. The groups of four larvae were then placed together in 50 ml tap water and monitored every 30 minutes to note interactions. In total, 24 groups of 4 larvae were set up over a number of days, using larvae of roughly the same size and within the age category of 9-11 days (but of the same age within a group).

The null hypothesis of no kin discrimination tested against is;

1 kin : 2 non-kin cannibalistic events (as larvae can not eat themselves)

Although all cannibalistic events were recorded in analysis, only the first has been used to test against the null hypothesis because subsequent events would not be independent of the first (for example, as one larvae would be occupied in feeding).

Chapter 3 Results

3.1 Experiment 1

3.1.1 Non-cannibalistic mortality

Control conditions of three first instars (replaced each day) were run for eight days during which time only 7 of 210 or 3.33% died. Non-cannibalistic mortality has therefore been excluded as negligible in the experiments and missing larvae attributed to cannibalism.

3.1.2 Fitness consequences of food and density levels

To expect deviation in importance of nutritional advantages and hence differences in cannibalistic rates, the levels of density and food used should represent limiting conditions for larval growth and development. Both conditions of low food and high density investigated had impact on the selected parameters of fitness.

Experimental treatments display that density and food levels affected survivorship. In the normal conditions 86.67% eclosed, in high density, 63.33% and in low food only 30% eclosed. As only three adults emerged from low food conditions, effects on development time and adult size could not be assessed. Density had a significant effect on both the size and development time of emerging adults Larvae took longer to develop and achieved smaller size in high density situations (median pupation time of 3 days and 4 days and medians of 2.46 mm and 2.17 mm for males and 2.93 mm and 2.53 mm for females, in normal and high density treatments respectively. Mann Whitney u values of 3.4750, p = 0.0005 and -2.7511, p = 0.0059 for male and female sizes and -3.2955, p=0.0010 for development time). Sexes have been considered separately for adult size as a significantly effect on size was found in both density levels due to females being larger in size than males (Kruskal Wallis $\chi^2 = 9.1000_1$, p=0.0026 and 2.4830_1, p=0.0130 for normal and high density respectively). Sex was not significant in explaining variation in pupation time however (Kruskal Wallis $\chi^2 = 0.4573_1$, p=0.4989 and 0.823_1, p=0.7718).

In addition, at high density, a significantly higher proportion of emerging adults were male (14 : 5), ($\chi^2 = 4.26321$, p = 0.0389) as opposed to 7 : 6 ratio in normal conditions, possibly due to females failing to emerge.

These results collectively prove that levels of food and density chosen did have fitness consequences, even with the ability to cannibalise. Similar limiting roles of density and food have been recorded for other species, (see Renshaw *et al.*, 1994 & Renshaw *et al.*, 1993 for *A. cantans*, Miller *et al.*, 1994 for *Wyeomyia smithii* (Coq), Williams, *et al.*, for *Culex* species, Jones, 1993 for *T. splendens*, Broadie & Bradshaw, 1991, Hard *et al.*, 1993, for *A. sierrensis*).

3.1.3 Expression of the cannibalistic trait

As total and rates of cannibalism in all conditions were not distributed normally, non-parametric statistical tests have been used.

In all cases total cannibalism and rates of cannibalism in high density treatments has been taken as an average of the number of large instars present in a cell as cannibalism can not be attributed to specific individuals. This may reduce variance in cannibalistic rates within high density situation. As the data is not distributed normally, this effect can not be tested for.

The proportion of individuals expressing a cannibalistic tendency varied between treatments, being highest in the low food treatment, where all individuals cannibalised and lowest in normal conditions, where 66.67% cannibalised. The high density treatment fell in-between these two extremes with 76.67% cannibalising.

To compare daily rates of cannibalism between treatments, only the first three, pupation-free days have been used. Significant difference in cannibalism rates over the first three days were found except between normal and high density, due to high rates of cannibalism in low food conditions, very low rates in high density treatments and intermediary rates in normal food (medians of 0.84, 0.11 and 0.33 first instars per day respectively and Mann Witney u values of 56, 2-tailed p = 0.2665 between density levels, 30.5, 2-tailed p = 0.0107, between food levels and 16, 2-tailed p = 0.0084 between low food and high density). Although rate of cannibalism was lower in higher density, total cannibalism per individual was greater than normal conditions. This could be a result of longer pupation time providing greater opportunity to cannibalise. In both cases Kruskal Wallis ANOVA tests showed no significant effect of sex on rate of cannibalism over the first three days (χ^2 of 1.0612_{1}, p = 0.3029 and -1.0734_{1},$_{,,}$ p = 0.2831 respectively).

This data shows that the variation in density and food effects both the occurrence of and rates of cannibalism.

Table 2 below, summarises the effect of treatments on adult fitness and expression of the cannibalistic trait;

Table 2; Summary of effect of treatments (density and food) on adult fitness parameters and occurrence of cannibalism

rearing condition	*survivorship*	*median length of hind tibia (mm)*		*median pupation time*	*sex ratio*		*% cannibalistic*	*rate of cannibalism (first 3 days)*
		male	*female*	*(days)*	*male*	*female*		
normal	86.67	2.46	2.93	3	7	6	66.67	0.84
high density	63.33	2.17	2.53	4	14	5	76.67	0.11
low food	30	-	-	-	-	-	100	0.33

3.1.4 Fitness consequences of cannibalism

A preliminary study (due to small sample sizes) of fitness consequences, by comparing rates of cannibalism within treatments with development time and size of adult, reveals no reproductive advantage.

Cannibalism may provide a nutritional benefit that shortens development time (and so decreases time to pupation), increases longevity in those that failed to pupate in high density and low food situations, or increases size. Spearmans rank coefficients only showed a correlation between total number of first instars consumed and longevity and pupation in high density ($r_S = 0.8013$, $p=0.005$ and $r_S = 0.7655$, $p = 0.000$ respectively), which probably only reflects the longer period of time to cannibalise as no significant correlations were detected between rates of cannibalism and time to pupation in normal or high density treatments, (p values of, 0.812, 0.249 respectively). No advantage to longevity in limiting conditions was detected as no correlation was found between rate over the first three days or overall rate and longevity in high density or low food conditions (r_S values of -.5, $p = 0.667$, 0.4025, $p = 0.249$, 0.114, $p = 0.807$, -0.260, $p = 0.574$ respectively) or between total cannibalism and longevity in low food treatments, ($r_S = .3077$, $p = 0.502$). Scatter plots of longevity/pupation time against rates of cannibalism over the first three days reveal no relationships (figures 1 - 3).

Size of adult, indicated by hind tibia length, was not correlated to rate of cannibalism over the first three days or overall or to total cannibalism, in either normal or high density situations (r_S values of -0.4062, $p = 0.168$, 0.068, $p = 0.521$, 0.487, $p = 0.092$, 0.132, $p = 0.601$, 0.261, $p = 0.28$ & 0.093, $p = 0.704$ respectively).

In high density treatments, to see if cannibalism provided a benefit to survivorship, rates of cannibalism over the first three day period were compared between pupating and non-pupating individuals. No difference was found between the two groups (z value of -1.1748, p = 0.2401).

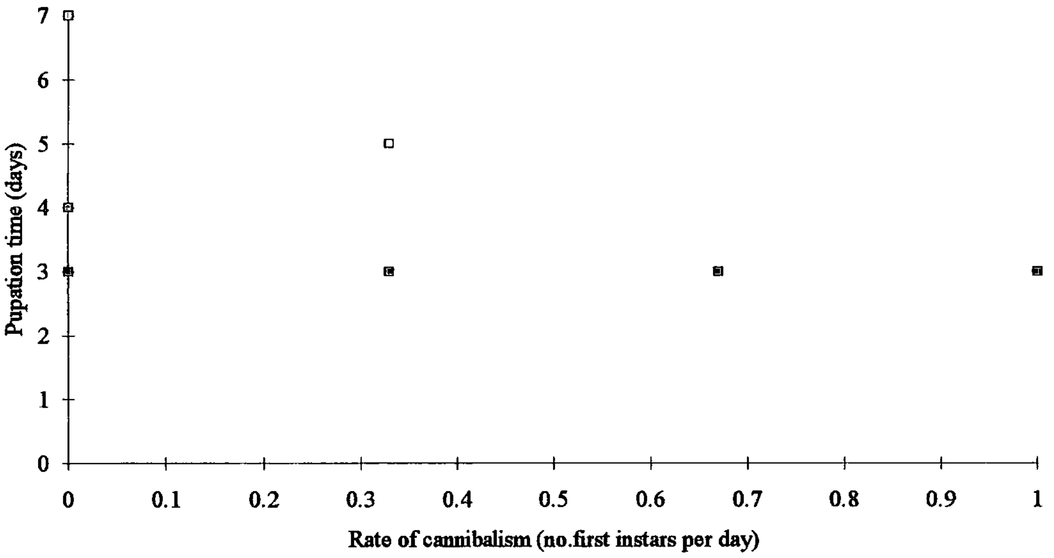


Figure 3.1; Experiment 1: Plot of pupation time in *A. aegypti* against rates of cannibalism over the first three days in normal conditions

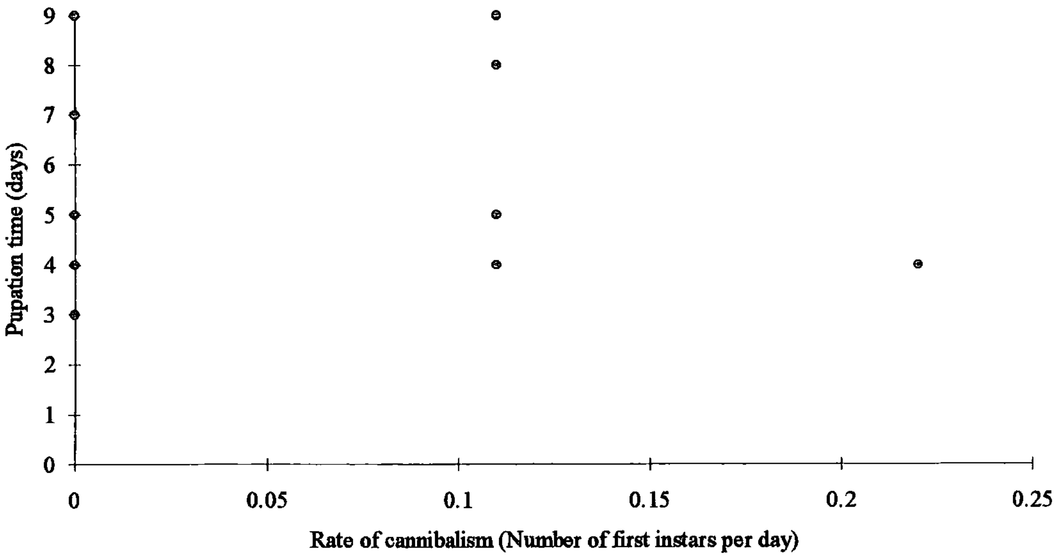


Figure 3.2; Experiment 1: Plot of pupation time in *A. aegypti* against rates of cannibalism over the first three days in a high density situation

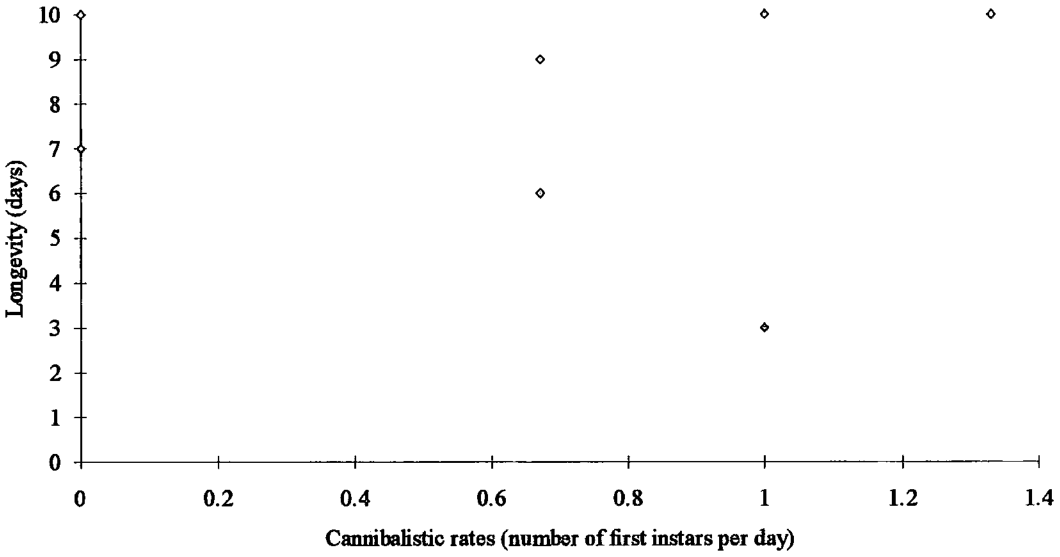


Figure 3.3; Experiment 1: Plot of longevity in *A. aegypti* against rates of cannibalism over the first three days in a low food situation

3.1.5 Role of physical interference to cannibalism

In high density treatment, a significant increase in cannibalism rates after first pupation was evident (see figure 4), (Wilcoxon matched pairs z value of -2.3664, 2-tailed p = 0.0180). As cannibalism rates had no significant effect on development time and sex did not effect rate of cannibalism, this relationship can not be an artefact of either factor.

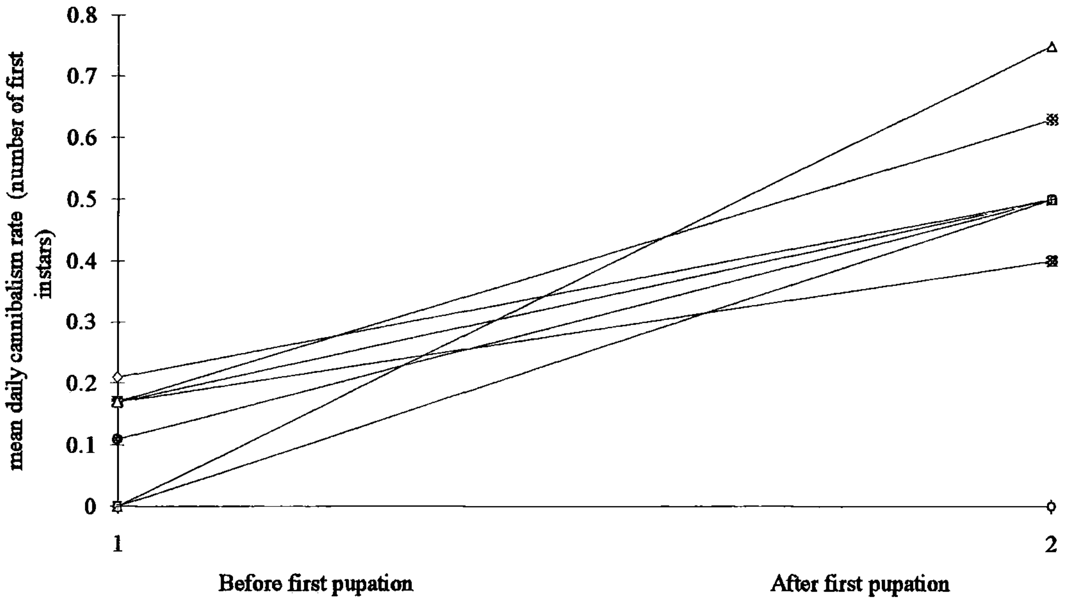


Figure 3.4; Experiment 1: Average daily rates of cannibalism by *A. aegypti* in high density conditions, before and after first pupation

3.2 Experiment 2;

Time to emergence and size of emerging adult were considered as indicators of a direct reproductive benefit of cannibalism in *T. digitatum*, (survivorship was almost entire in both conditions). These variables were compared between those individuals given an opportunity to cannibalism and those that were not and within cannibals to see if rate of cannibalism (number of first instars eaten per day) correlated with variation in size or time to pupation.

Hind tibia lengths, cannibalistic rates and emergence times in both sexes are normally distributed hence parametric statistics have been used in this analysis.

Although the experiment would appear on first sight to be hierarchical in design, with opportunity to cannibalise and sexes nested within clutches, all data points (individuals) are independent and as individuals within a clutch are not numbered, utilisation of a factorial design will not cause any false correspondence between individuals among clutches. However as the clutches represent a random sample from natural populations and inferences will be extended to populations in general, clutch has been considered a random effect and hence a mixed model ANOVA employed. This means that, in contrast to a fixed model, the within cells error term mean square is used as denominator for the f-ratio, only where the main effect or interaction tested includes a random variable, otherwise the appropriate interaction mean square is used as denominator, (refer to Zar, 1984, page 474, Sokal & Rohlf, 1987, chpters., 8 - 11)

Inherent non-orthogonality means that order in the table is important. Therefore sequential sums of squares have been used to compose mean squares, whereby significance of variability explained by one x variable will be considered when only variability accounted for by the x factors higher up the table (as opposed to all x variables in adjusted sums of squares) have been excluded. This ensures that main effects are not tested using a sums of squares that has been adjusted for an interaction involving the main effect, hence conforming with the law of marginality.

3.2.1 Fitness comparisons between cannibals and non-cannibals

Opportunity to cannibalise does not explain a significant amount of variation in either size or emergence time (ANOVA f-values of $3.76_{1,15}$, and $2.59_{1,15}$,, $p > 0.05$ respectively). Sex of the individual was found to be a significant factor to both size and emergence times in both cannibals and controls, due to larger size of females (mean hind tibia length of 2.77 mm as opposed to 2.65 mm) and protandry, (mean female emergence times of 14.3259 compared to 13.403 days for males), (ANOVA f-value of $85.44_{1,15}$, $p = 0.000$ and $32.92_{1,15}$, $p < 0.01$ respectively). There were no significant interactions.

3.2.2 Fitness comparisons within cannibalistic individuals

No linear correlation exists between rates of cannibalism and size in either males or females (Pearsons 2-tail significance values of 0.341 and 0.946 respectively). Scatter plots, (figures 3.5 and 3.6), show no higher order or non-linear relationships. Plots of emergence time against rate of cannibalism (see figures 3.7 and 3.8) show no correlation in either sex. In addition, there was no confounding relationship between pupation time and size of adult (see figures 3.9 and 3.10).

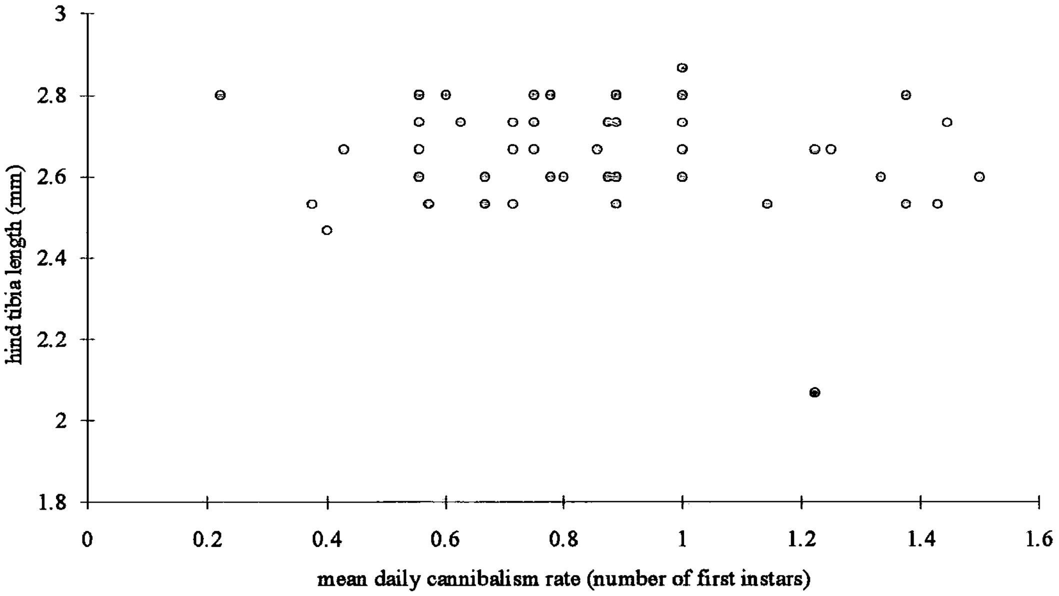


Figure 3.5; Experiment 2: Plot of hind tibia length of male *T. digitatum* against mean daily rate of cannibalism

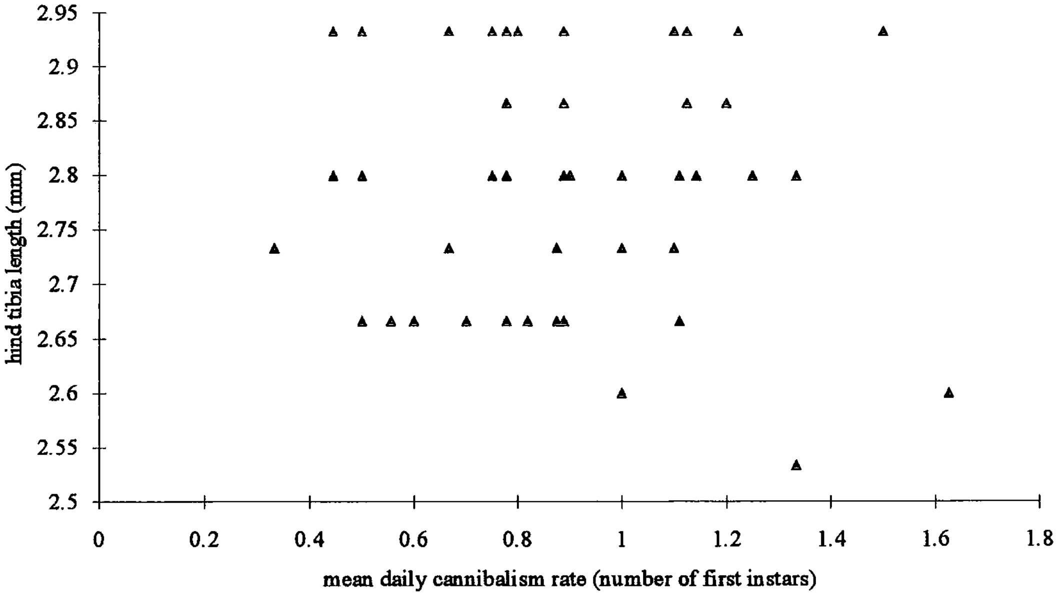


Figure 3.6; Experiment 2: Plot of hind tibia length of female *T. digitatum* against mean daily rate of cannibalism

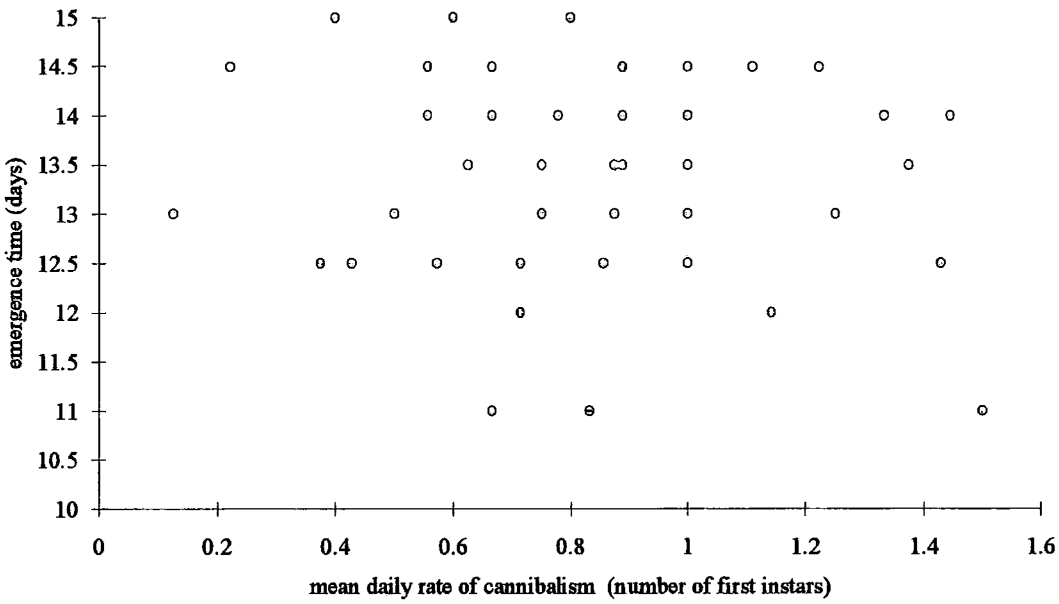


Figure 3.7; Experiment 2: Plot of *T. digitatum* male emergence time against mean daily rate of cannibalism

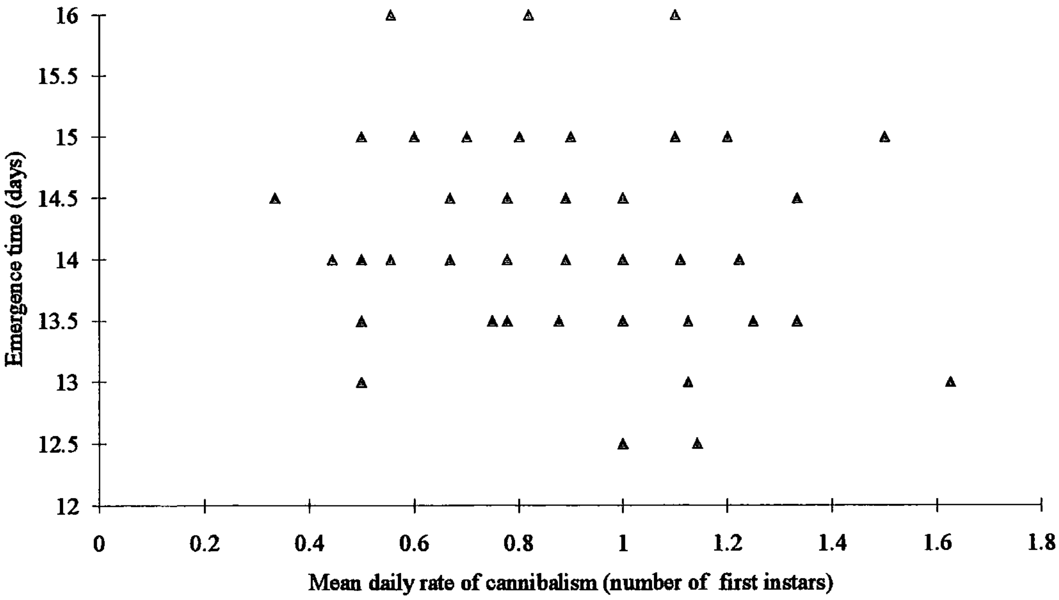


Figure 3.8; Experiment 2: Plot of *T. digitatum* female emergence time against mean daily rate of cannibalism

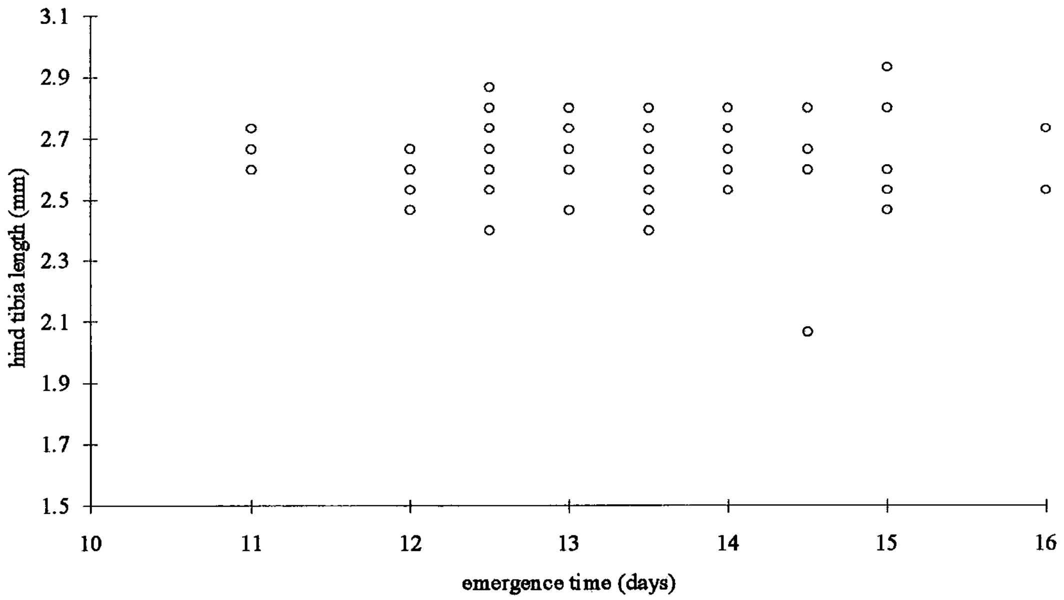


Figure 3.9; Experiment 2: Plot of hind tibia length of male *T. digitatum* against emergence time

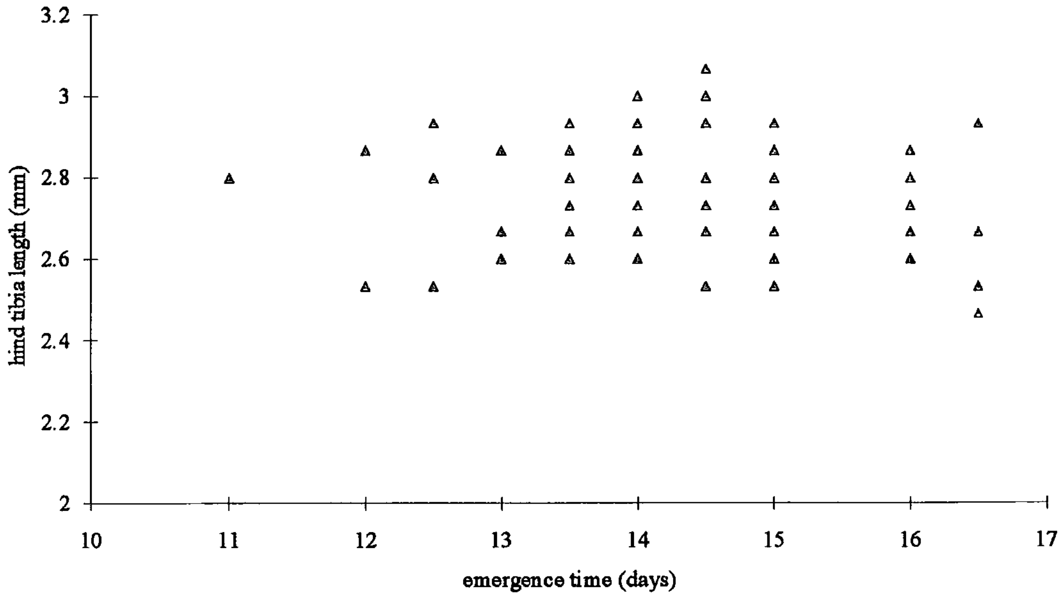


Figure 3.10; Experiment 2: Plot of hind tibia length of female *T. digitatum* against emergence time

3.2.3 Inter-clutch variability

Cannibalism rates are significantly different among clutches (f-value of $1.84_{15,15}$, p = 0.041). Sex was not a significant factor, (f-value of $1.67_{1,15}$, p>0.05). As there was also no significant interaction between clutch and sex, i.e. one did not modify the effect of the other on cannibalistic rates, sexes have been amalgamated to show inter-clutch variability in mean cannibalism rates (Figure 3.11).

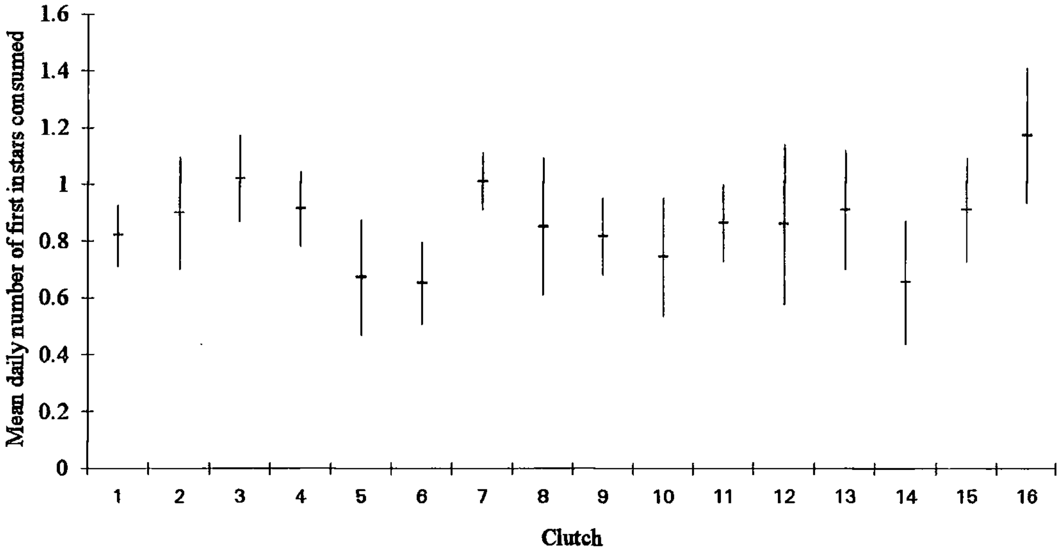


Figure 3.11; Experiment 2: Mean (and confidence of mean) daily cannibalism rates of *T digitatum* for each sex from each clutch

Clutch also proved significant in explaining variation in emergence time, (f value of $3.46_{15,178}$, p=0.00) and adult size (f = 4.07, p = 0.000). Figures 3.12 - 3.15 show mean (and confidence limits of mean) emergence time and hind tibia lengths among clutches, with sexes displayed separately due to its' significance in explaining variability of these parameters. As opportunity to cannibalise was not a significant factor to either variation in emergence time or size, cannibals and controls have been combined to provide a clearer picture. Comparison between graphs support the following putative trends; variation in tibia size among clutches is similar for both sexes but with a particularly close, positive association between female tibia length and rate of cannibalism, suggestive of a slight but insignificant fitness advantage. Variation in emergence time is comparable between the sexes. The significant effect on emergence time could be an artefact of inter-clutch differences in hatching times.

However, within the same clutch, cannibal and control mean emergence times do not collate, presented by figures 3.16 and 3.17. Consequently, no obvious explanation for variation in emergence time exists.

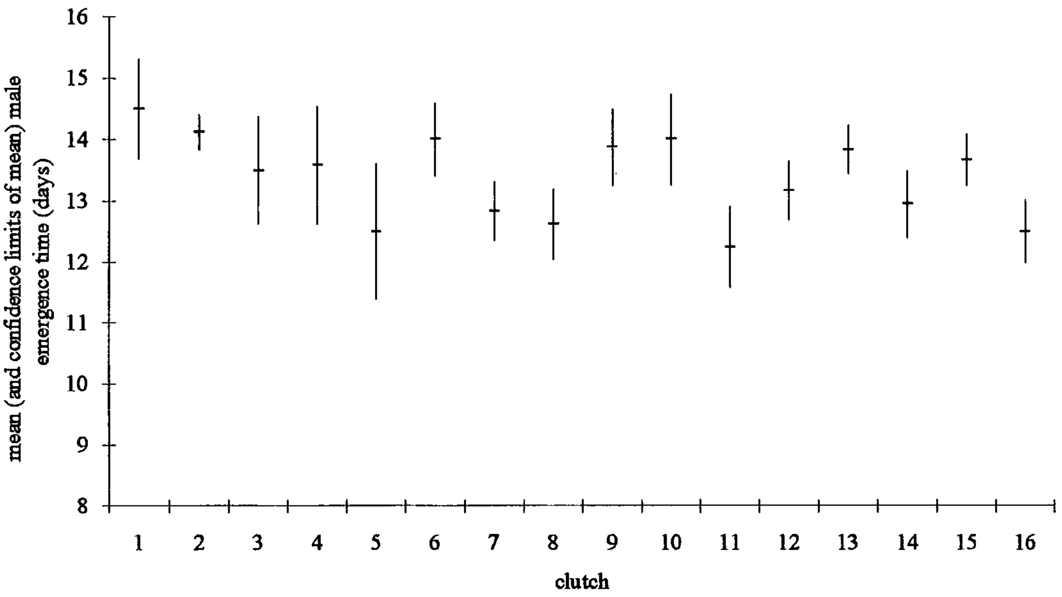


Figure 3.12; Experiment 2: Mean (and confidence of mean) emergence times of male adult *T. digitatum*, from each clutch

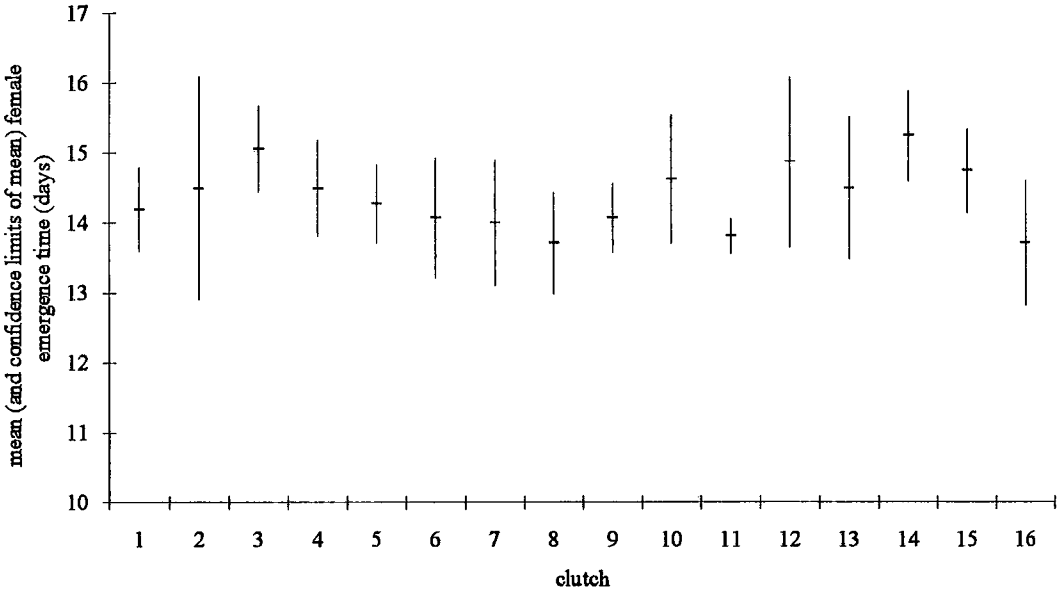


Figure 3.13; Experiment 2: Mean (and confidence of mean) emergence times of female adult *T. digitatum*, from each clutch

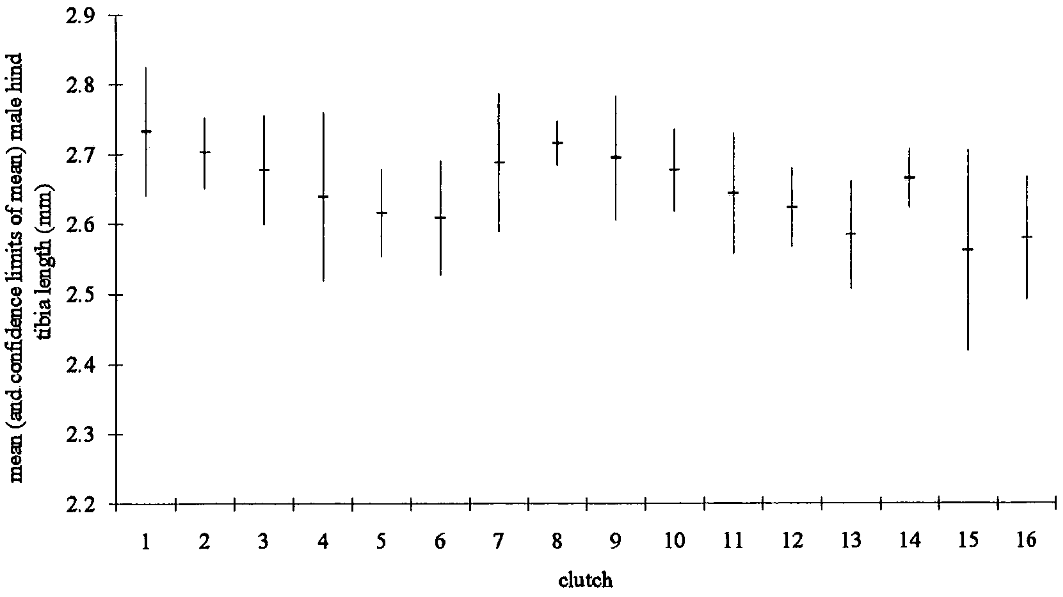


Figure 3.14; Experiment 2: Mean (and confidence of mean) hind tibia length of adult, male *T. digitatum*, from each clutch

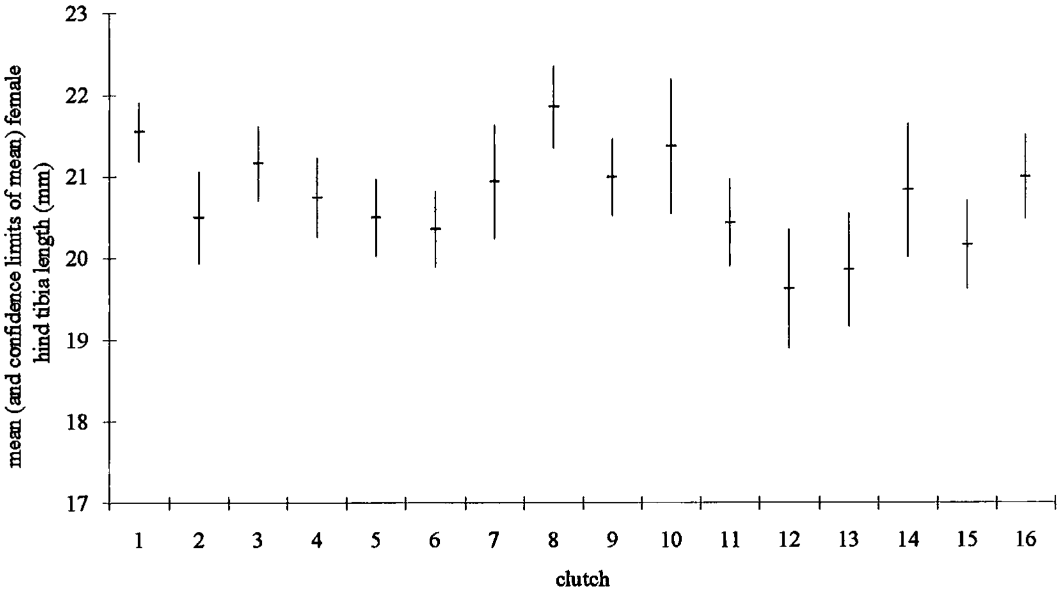


Figure 3.15; Experiment 2: Mean (and confidence of mean) size of adult, female *T. digitatum*, from each clutch

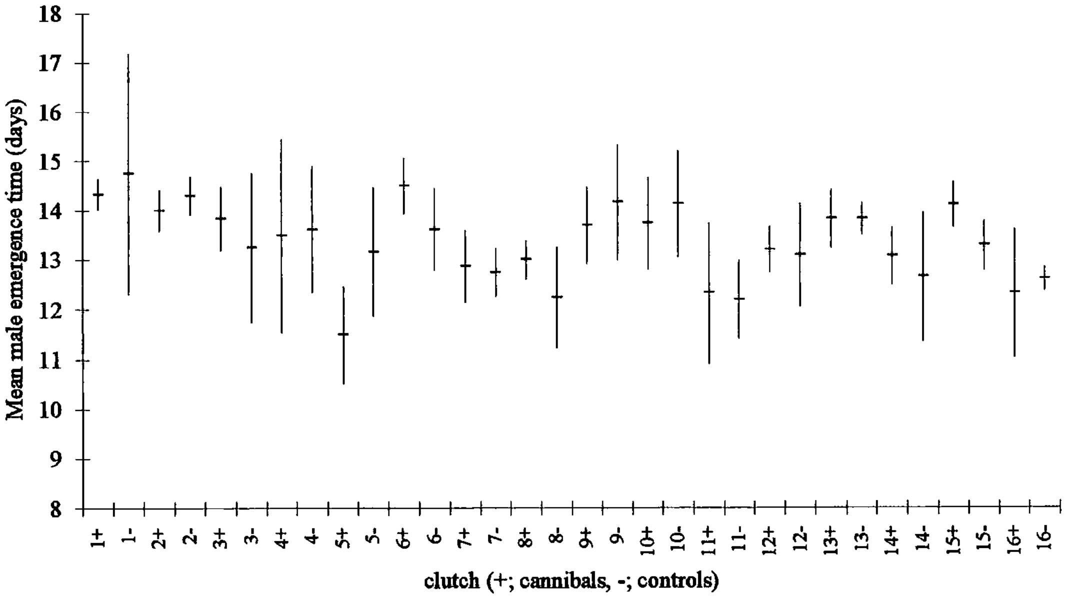


Figure 3.16; Experiment 2: Mean (and confidence of mean) emergence times of adult male *T. digitatum* cannibals and controls, from each clutch

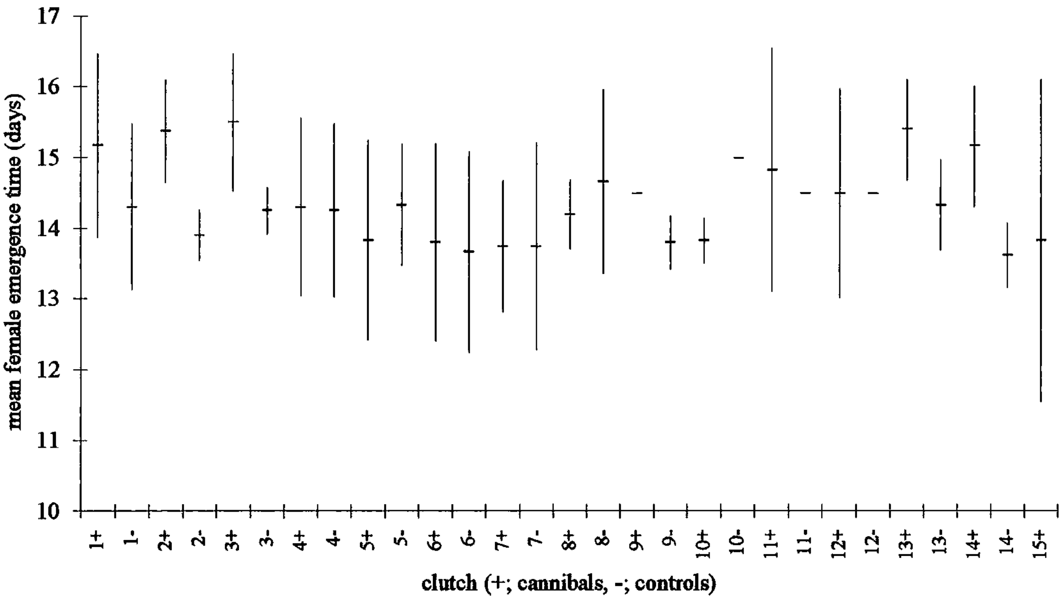


Figure 3.17; Experiment 2: Mean (and confidence of mean) emergence times of adult female *T. digitatum* cannibals and controls, from each clutch

Table 3; Summary of ANOVA significance values (p = 0.05) of x variables, and their interactions, to explaining variation in adult size, development time and rate of cannibalism in *T. digitatum*

x variables	***y variables***		
	size	*development time*	*rate*
cannibalism	X	X	X
sex	✓	✓	X
clutch	✓	✓	✓
all interactions	X	X	X

Table 4; Summary of significant correlations within larvae of *T. digitatum* given the opportunity to cannibalise

variables	*size*	*development time*
cannibalism rate	X	X
development time	X	-

3.3 Experiment 3

3.3.1 *C. mollis*;

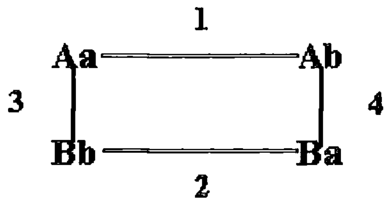
As only 5 of 360 or 1.39 % of non-kin and 3.61 % of kin first instars disappeared, it can be assumed that in the conditions provided, *C. mollis* does not demonstrate a cannibalistic tendency, irrespective of relatedness.

3.3.2 *T. digitatum.*

3.3.2.1 Comparison of cannibalistic rates in kin and non-kin situations

Cannibalism occurred frequently and in all replicas, with means of 5.4 and 5.7 individuals out of 12 consumed between day three and pupation in non-kin and kin treatments respectively.

Once again number consumed was normally distributed and so parametric tests apply. The pairwise nature of the experiment entails not only cannibalism within kin and non-kin for each clutch to be considered but also within pairs of clutches, for propensity to cannibalise and evasion of cannibalism to be compared between clutches, as shown below;



Where A and B are separate clutches, small case represent prey and capitals, cannibals. Inter-clutch differences in cannibalism can be tested by considering ratios of cannibalism depicted by 3 and 4 as, in each of these, relatedness between cannibal and prey are the same (either both kin or non-kin). Pairs will be discounted from analysis of relatedness firstly if large instars of A and B differ more than 25 % in the total number of prey eaten in "3" and "4" together and secondly by a 10 % cut-off level This excludes the following data points, denoted "*" and "**" (additional exclusions for the second case) in table 5 below. Data for two sets of clutches had to be abandoned due to accidental spillage, leaving twenty six clutches.

Table 5; Experiment 3: Across pairs comparison of cannibalistic tendencies (number of prey eaten) in *T. digitatum*

Aa : Bb	**Ab : Ba**	**Total**	**smallest/largest**
4 : 7	6 : 7	10 : 14	0.71 *
8 : 7	5 : 11	13 : 18	0.72 *
5 : 4	2 : 3	7 : 7	1
9 : 1	3 : 11	11 : 12	0.92
6 : 2	5 : 2	11 : 4	0.36 *
2 : 6	6 : 2	8 : 8	1
9 : 8	9 : 3	18 : 11	0.61 *
6 : 4	4 : 5	10 : 9	0.9
2 : 6	4 : 1	6 : 7	0.86 **
11 : 7	3 : 9	14 : 16	0.88**
6 : 6	10 : 7	16 : 13	0.81**
5 : 5	2 : 10	7 : 15	0.47 *
1 : 3	2 : 6	3 : 9	0.33 *

Degree of cannibalism between kin and non-kin treatments does not significantly differ. This procedure provides a robust test as the same conclusion holds, whatever the degree of stringency, (Goodness of fit coefficient of 41.2128_{26}, $p>0.05$ for all clutch pairs and 28.766_{13}, $p>0.05$ and 19.049_{7} $p>0.05$ for clutches filtered at the 25 and 10 % levels respectively).

3.4.2.2 Effect of sex on cannibalism between kin and non-kin;

As differences in tendencies to cannibalise have, as previously mentioned, been noticed between males and females, possibly due to varying nutritional requirements, it may be expected that costs and benefits of kin cannibalism vary also, (for example, Agarwala & Dixon, 1993).

Where all four potential cannibals emerged, percentage eclosing as female has been plotted against number of "prey" consumed (see figures 3.18 and 3.19) for kin and non-kin treatments. Where "prey" were kin the number of female cannibals clearly had no effect on the number eaten (Regression F-value of $0.4714_{1,}$ p = 0.502). However when prey were not kin, number eaten increases with the proportion of female cannibals. This proved a statistically significant regression (F-value of $5.273_{1,}$, p = 0.047) although sample size is noticeably small and fit, far from perfect.

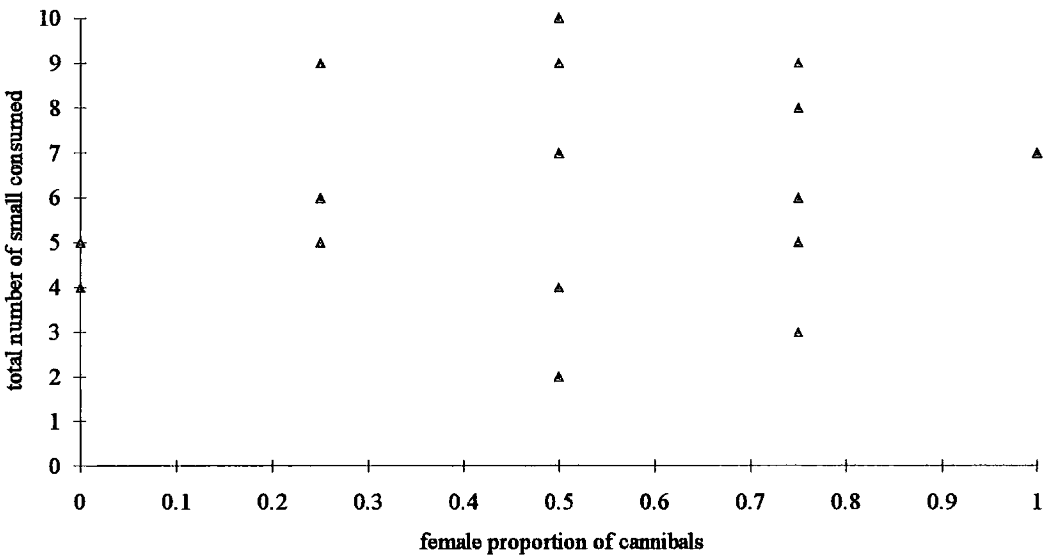


Figure 3.18; Experiment 3: Plot of number of small, kin instars consumed against female fraction of cannibals in *T. digitatum* where all cannibals eclosed

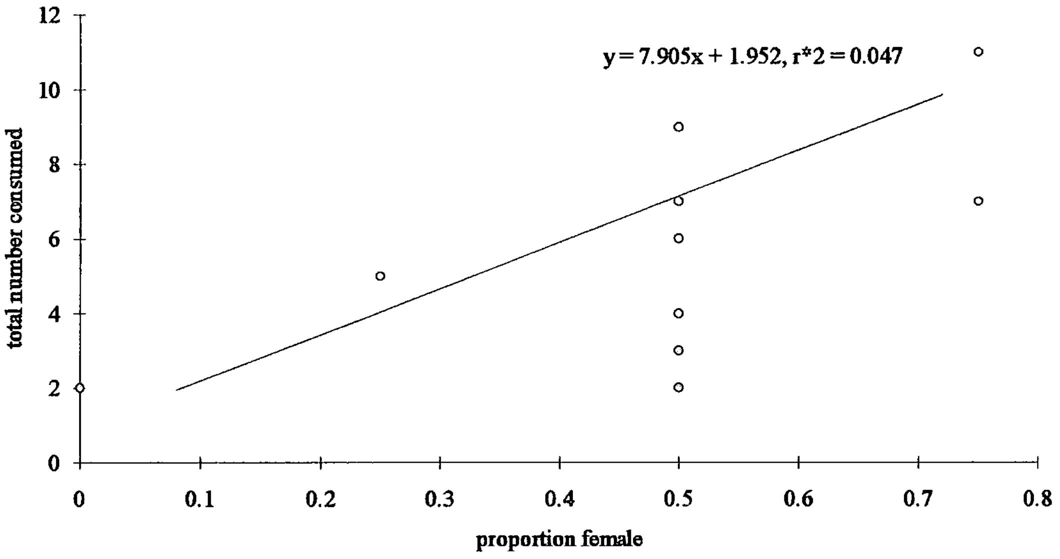


Figure 3.19; Experiment 3: Plot of number of small, non-kin instars consumed against female fraction of cannibals in *T. digitatum* where all cannibals eclosed

3.4.3 *Toxorhynchites moctezuma*

3.4.3.1 Frequency of kin versus non-kin cannibalism

In 15 cases non-kin were chosen as prey and in 6 cases kin were selected. This distribution of first choice cannibalism between kin and non-kin does not differ from that expected by chance, Binomial test, (P (n = 15), Bin (21, 0.667), one tailed significance of 0.4314).

3.4.3.2 Confounding variables

To ensure that kin discrimination has not been masked, two additional factors must be controlled for, larval size and group size. Although individuals with the smallest size range were chosen from each clutch, some variation inevitably existed, especially between the first and second individual of each clutch. However when individuals within each group of four are ranked from smallest to largest, the distribution of difference in ranks between the first choice cannibal and victim over all groups of four does not differ from random, (Goodness of fit $\chi^2 = 5.1429_5$, p = 0.7422). Size of cannibal, size of victim or rank differences between cannibal and kin size do not vary between kin and non-kin cannibalistic events (Mann Whitney u values of -0.9532, p = 0.3405, -0.7856, p = 0.4321 and -0.8255, p = 0.4091 respectively). This suggests that there is no significant, confounding size bias, for example of the cannibal always being the largest and the victim the smallest, as displayed by the distribution of sizes of cannibals and victims in figure 3.20.

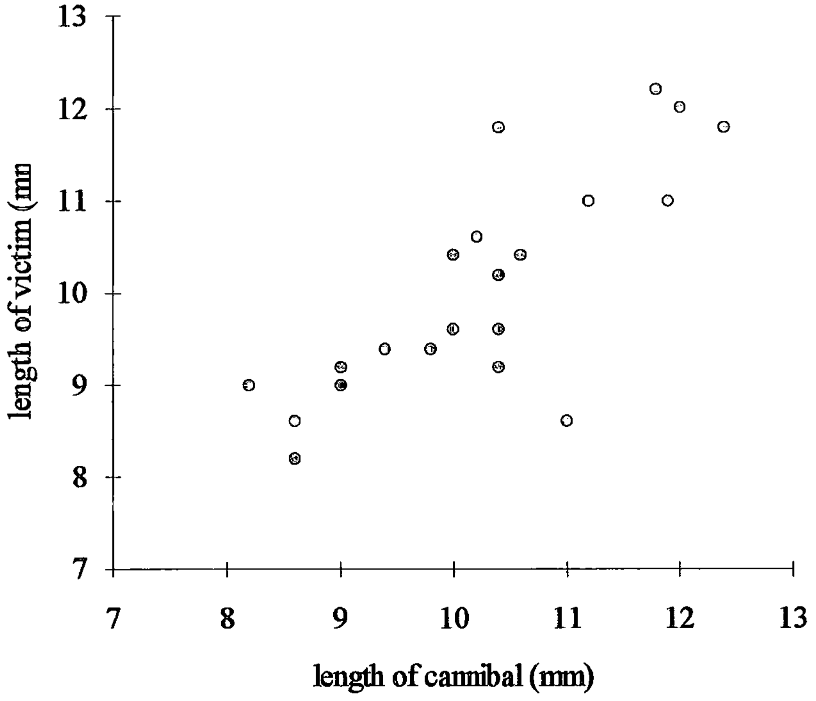


Figure 3.20; Experiment 3: Scatterplot of length of victim against associated cannibal length in *T. moctezuma*

Although clutches of eggs greater than 10 were ignored, there is still a chance that eggs have been laid by different females. This probability will presumably decrease with clutch size (as two females are less likely to both lay one egg). Comparison of clutch sizes between kin and non-kin cannibalistic events show no significant difference (Mann Witney u-value of -0.953, 2-tailed p = 0.34 between kin and non-kin cannibal clutch sizes) and no correlation was found between difference in ranks and cannibal group size ($r_S = 0.0611$, p = 0.793).

Chapter 4 Discussion;

4.1 Nutritional benefits

4.1.1 *A. aegypti;*

The levels of density and food used had obvious fitness consequences for *A. aegypti*, in terms of survivorship, development time and size of emerging adults, with high density and low food levels increasing probability of larval mortality, prolonging development (and in the case of low food, preventing pupation) and reducing adult size. In both cases, therefore, the nutritional benefits accrued from cannibalism may be expected to be more significant and hence for cannibalism to be expressed more frequently and at a higher rate. Not only was fitness of *A. aegypti* adults reduced at high larval densities but also, females were significantly under represented amongst emerging adults when compared to the 50 : 50 sex ratio of normal conditions, suggesting over-representation of females in larvae that failed to complete development. This skewed sex ratio supports differentiation of nutritional requirements, further backed by the larger size of females. Additional costs of blood host searching and oogenesis may entail a higher threshold weight for pupation in females. It would therefore be expected, if dynamics of cannibalism are regulated by nutritional advantage, that in addition to between treatment differences, females should cannibalise at a higher rate.

Comparison over the first three days does in fact show that occurrence and rate of cannibalism is higher at lower food supply. However, contrary to predictions, rate decreased with increasing density. This is supported both by comparison between normal and high density situations and comparison before and after the first pupation at high density. As *A. aegypti* filter feed by creating feeding currents, reduced cannibalism is likely to have been caused by physical interference between large larvae, (a role of physical interference has been noticed in *A. sierrensis* at 64 larvae/ml, Broadie & Bradshaw, 1991) These findings put to question the theory that

> "facultative cannibalism might have been a strong factor in the evolution of egg hatching inhibition"
>
> (Koenekoop & Livdahl, 1986),

suggesting that avoidance of the deleterious fitness consequences of high density due to physical interference is likely to have been a more significant evolutionary pressure.

Despite differences in incidence and rates of cannibalism among treatments, cannibalism did not compensate for limiting conditions, in terms of the fitness parameters measured. A number of likely factors could contribute;

1. At low food levels the same reasons suggested previously for *A. triseriatus* and supported by low survivorship in *T. digitatum,* could apply.

2. At high density the constraining role of physical interference limiting cannibalism precluded any compensatory increase in rate of cannibalism.

3. By replacing first instars each day, a conservative measure of natural difference in rate of cannibalism between high and low food and density situations has been achieved by artificially increasing cannibalism in normal conditions and suppressing it in limited conditions. The consequential reduction in range of cannibalism could have obscured identification of fitness advantages. Together with the shortage of resources experienced by the potential cannibal, limited resources cause restricted growth in first instars making them more vulnerable to predation for a longer period of time (particularly important to highly size dependent cannibalism), so influencing the potential predator : prey densities within populations. Pertinent studies include the finding that young larvae of the aquatic *Saduria entomon,* were vulnerable for three times longer in deeper, food-limited zones than those in food-rich shallow zones (Leonardsson, 1991). Also at high levels of hunger, small larvae are more likely to adopt risky behaviour to forage as elevated nutritional benefits will outweigh higher costs incurred through risk of predation. *A. triseriatus* larvae adopted more risky browsing behaviour in the presence of *Toxorhynchites rutilus* with increasing hunger, (Juliano *et al.*, 1993).

4. Associated costs of cannibalism, for example, of eating kin (highly likely as laboratory strains are often highly inbred), group size reduction or risks of retaliation may outweigh nutritional benefits at higher rates of cannibalism, preventing complete compensation for limited resources (costs rising through increasing probability of eating kin and reduction of group size and potential mates) or make cannibalism a less energetically efficient food type.

5. If cannibalism is purely facultative, with adjustment in filtering rate in different ecological conditions accounting for variation in cannibalistic rates between treatments, an upper limit on filtering rate could constrain nutritional compensation at low food. Opportunity to cannibalise may well have improved longevity.

Both of the latter two factors could explain the lack of correlation between fitness and rate of cannibalism within a treatment. However, what of the seemingly paradoxical lack of sex differences in cannibalism rates ?

In conclusion, variation in expression and rate of cannibalism with ecological condition provides evidence for a role of nutritional benefits as a selective force. However although sample sizes were small and many possible, unidentified fitness parameters exist, the anomalies mentioned above suggest that relative nutritional benefit is not the only factor determining the selective advantage of cannibalism to reproductive success. Cannibalism could procure fitness benefits through limiting physical and chemical interference or elimination of potential competition for mates or be constrained for reasons suggested in point 4. Larger scale studies, evaluation of mating success on emergence, manipulation of proposed costs and comparison to autogenous species such as *T. moctezuma* would be informative in determining the relative selective pressures of these factors.

4.1.2 *T digitatum;*

Contrary to expectations, study of cannibalism, at intermediate food supply in *T. digitatum*, revealed no fitness consequences in terms of development time and adult size both when cannibals were compared to controls and within cannibals when differences in rates of cannibals were compared to fitness parameters. Also, in contrast to previous findings at high food (Sherratt & Church, 1994), no consistent differences in propensity to cannibalise existed between males and females. It is tempting to hypothesise that, whereas at high food males, due to a lower nutritional demand, have sufficient food for development without cannibalising, at intermediate food levels, cannibalism can provide a nutritional advantage, so eliminating sex differences. However, as size is not correlated with opportunity to cannibalise in either sex, this cannot apply.

Want of differences in fitness between cannibals and controls suggests that larval development was not limited in this food condition. The extra energy required to capture smaller conspecifics, or the competition for limited food that they consequent, may balance out or reduce nutritional benefits when compared to passive feeding on detritus, explaining why cannibalism provides a nutritionally-derived fitness advantage in *T. digitatum* only when other food supplies are insufficient. Occurrence of cannibalism at higher food levels could simply be a by-product of nutritional benefits to fitness in scarcity of food. Alternatively advantages may be reflected in unmeasured parameters such as shortening generation time by aiding egg development.

Occasion of cannibalism where it appears to have no measured fitness reward and disparity of sex effects between food levels could alternatively suggest that, once again, other factors must be considered to explain when cannibalism is advantageous to reproductive success.

It is important to stress that food conditions used in this experiment were not strictly intermediary to those used previously as food was provided in instalments. This could have influenced the nutritional demands on cannibalism.

4.2 Heritability of cannibalism

The existence of genetic variability in expression of the cannibalistic traits demonstrated between clutches of *T. digitatum* proffers an inherited predisposition to cannibalistic trait expression. This not only provides some evidence for a genetic base and hence an opportunity for an adaptive role of cannibalism, but also eludes to its' selective nature. Usually two reasons are proposed to explain variability, multiple adaptive peaks or selective neutrality. As individuals were all collected from the same locality, local adaptive peaks can not be proposed to explain variability. Therefore, variability should indicate that cannibalism is selectively neutral (as proposed for genetic variability among laboratory *Tribolium* strains, when cannibalistic rates were stable for 60 generations, (Stevens, 1989)) as if of selective advantage, variation would be eliminated through natural selection. There are two main reasons why this does not necessarily have to follow in this case;

1. Existence of a gene-environment interplay entails behavioural patterns rarely precisely mapping genes.

The heritability component of satellite versus caller strategies in male field crickets, for example, has been estimated at about 50 % (Caro & Bateson, 1986). Variability in fitness can be maintained by virtue of its low heritability. Although genetic variability exists, control by many heterogeneous environmental factors may mean that it is rarely expressed, probably only in habitats perceived as homogeneous, a quality perfected by this experiment. However, even in this case, inter-clutch differences could still be explained entirely environmentally for instance, by size of blood meal in the female affecting resources provided in a single egg (known to explain variability in egg and clutch size and hence larval size in *A. aegypti*, Hard & Bradshaw, 1993), so making explanations for genetic variability redundant. Therefore even if a heritable genetic component to propensity to cannibalise exists (which is highly questionable), cannibalism can still be of adaptive significance as such variability is rarely likely to be expressed in nature.

2. Inter-clutch variability as a product of frequency dependent selection., maintained as a mixed evolutionary stable strategy (ESS), (Maynard Smith, 1982).

Applicability of an ESS requires the benefits of cannibalism to depend on the cannibalistic behaviours employed by neighbours, producing inherited stable variation in trait expression. As pay-off to cannibalism would be expected to depend on proportional expression of the trait, with the higher the cannibalistic percentage of a population, the higher the potential costs of cannibalism due to increasing retaliatory risks and vice versa, the existence of a mixed ESS could possibly be envisaged within a single population. However, movement of ovipositing females between tree-holes would make it unlikely that offspring will experience neighbours with the same cannibalistic tendencies as the parents, so questioning the uninvaidability of an ESS between generations. Therefore reality of an ESS appears improbable except possibly in rare cases of perceived homogeneity of environment (for example, if female oviposition was site-faithful).

The logical next step would be to determine if the cannibalistic tendencies are heritable. This can be done by repeating the same experiment but in addition marking the emerging females, recapturing them with their offspring in the field, repeating the experiment on the offspring and testing for correlation of cannibalistic tendencies between mother and offspring. *T. digitatum* provides a rare opportunity for such an experiment by virtue of its' egg guarding behaviour.

4.3 The cost of eating relatives

Although species investigated varied with respect to expected benefits, and hence probability of, kin discrimination, empirical evidence suggests no manifest role of kin selection in cannibalistic behaviour within the species used.

This was as predicted for *T. digitatum,* due to the improbability of contact between relatives of large enough size difference to allow cannibalism to be beneficial. However the data implied an influence of sex of cannibal on kin discrimination as total cannibalism increased with proportion of female cannibals only in non-kin cannibalistic experiments. Firstly this correlation suggests that females may have a tendency to cannibalise more in low food (in contrast to the findings of Experiment 2, at higher food) and secondly that, as this only occurred in non-kin situations, that females may employ kin discrimination when cannibalising. It may be that larger adult size of females is reflected in larval size and/or that higher nutritional demands make it more likely that females could eat smaller, weaker individuals of the same clutch, hence increasing probability of kin cannibalism contact. A larger-scale study, with particular concentration on sex of cannibal and upper size-range of conspecifics consumed would be required to investigate these hypotheses.

C. mollis did not show any significant levels of cannibalism in either treatment. This leads to question why one tree-hole species should cannibalise but not another. It may be that experimental conditions employed did not limit development in *C. mollis*, especially likely as larvae were noticeably smaller in size than *T. digitatum*. Alternatively cannibalism may be constrained by a biological factor, for example the more evasive and faster movements of first instars (personal observation) or method of feeding (such as the previously mentioned a la carte and table d'hôte dichotomy).

Although the advantages of reciprocal altruism between kin during cannibalism would appear clear for *T. moctezuma* larvae, discrimination was not found in this study. A number of adaptive explanations why cannibalism could accommodate costs of eating kin are plausible.

- Nutritional advantage;

1. Predictability of food supply;

T. moctezuma differed from all other larvae by virtue of its' opportunistic predatory behaviour as opposed to feeding on detritus, characteristically a more variable food supply. Nutritional benefits accrued from cannibalism need not be so large to make cannibalism beneficial, due to unpredictability of food supply increasing risks of mortality and limiting development. These benefits are consequently more likely to outweigh cost to inclusive fitness of eating kin. Significantly, the only case of filial cannibalism documented in beetles occurs in the burying beetle which feeds on corpses that are unpredictable, scattered clumps of resources, (Bartlett, 1987). These greater benefits could also explain the predilection, present only in *T. moctezuma*, of the species studied and documented, to eat larvae of the same size or larger, as greater retaliatory costs could also be afforded. Frequent documentation of only one larvae surviving per pot provide support for such large benefits. However it has also been noticed that several larvae can coexist to pupation. It may be that these are conditions of abundant prey and that larvae assess their food supply before eating conspecifics. A parallel can be made to parasitoids in this respect where it is often more beneficial to kill competitors before they exhaust food supplies. Observation of *T. moctezuma* cannibalising in the presence of abundant alternative food (*T. digitatum*) furthers this analogy.

However, even if such an assessment is made, in terms of the cannibals' inclusive fitness, it would still seem preferable to eat non-kin first in case food conditions changed. Consideration of the behaviour of a potential victim in limited food is required to redress this anomaly. The victim would benefit its' inclusive fitness by sacrificing itself to a kin rather than non-kin cannibal, so cost of cannibalising kin could be reduced through lower risk of retaliation. This would be particularly true where larvae were of markedly different sizes/ health, increasing inequality of pay-offs in a prisoners dilemma game (by increasing b and decreasing c in the equation $rb > c$).

It would be interesting to test these ideas by completing similar experiments in conditions of varying alternative prey, stability of habitat and comparison to species living in more permanent habitats such as ponds and lakes

2. Physical interference of feeding;

Costs to food intake, through physical interference of the spatially-demanding feeding methods, (Steffan & Evenhuis, 1981, Linley & Darling, 1993, Linley, 1995) could outweigh costs of eating kin. However this is unlikely to be able to explain natural reduction to under five larvae per tree-hole.

- Genetic fitness;

Avoidance of deleterious genetic effects linked to inbreeding could be a governing factor, outweighing benefits of kin discrimination by providing an additional cost to avoiding relatives. Little is known of *T. moctezuma* mating, but if they mate immediately on emergence, the disadvantage of eating preferentially non-kin, in terms of inbreeding, are clear, (especially as, in the context of this study, *T. moctezuma* generally lays comparatively small clutches). Obviously clutch size and ovipositional tendency to lay where *T. moctezuma* eggs are already present will play significant roles by controlling relative numbers of kin : non-kin within a larval habitat and so warrant further study

- Mortality at pupal stages;

If the first larvae to pupate instinctively kills all others irrespective of their relatedness, due to high risks of pupal mortality, methods of kin discrimination during larval development would become rather redundant. Corbet and Griffiths concluded that, based on feeding rates on *A. aegypti*, compulsive killing in *T. brevipalpis* "could be expected to cause the death of all the other mosquito larvae in a tree-hole of average size" (Corbet & Griffiths, 1963). Therefore this premise is not biologically unfeasible

- Sexual selection;

Sex effects may actually mask kin discrimination. It is commonly found that, within a breeding population, the sex investing the least parental investment in offspring (normally the males due to anisogamy) experiences the most variable reproductive success and the other sex represents a fastidious rare resource to be fought over (initially ascertained by the classical work of Bateman on *Drosophila melanogaster* mating systems, Bateman, 1948). As deviations from a 1: 1 sex ratio imbalance the average reproductive success of each sex, they are generally evolutionary unstable and so cannot redress the balance (Fisher, 1930). It can be envisaged that competition could commence in larval stages of mosquitoes, in the form of cannibalism, with higher probability of intra-sexual as opposed to inter-sexual conflicts, particularly between males and that this is reflected in first choice cannibalism. Unfortunately, with the experimental design followed, it was impossible to identify sex of victims as larvae cannot be sexed and it is not known whether larvae can distinguish gender.

- Inter-clutch variation in relatedness;

Experimentation for the role of relatedness in explaining occurrence of cannibalism assumed that, in the case of *Toxorhynchites* (also applicable to other species laying single eggs) eggs laid in one pot were more genetically related than between pots (the basis for kin and non-kin distinction). Although frequent collection of eggs attempted to control for this, personal observation revealed that more than one female could lay in the same pot within a half hour period. Whilst average relatedness should still be higher overall within "clutches" than among, substantiation of this assumption through genetic analysis would be a clear advantage.

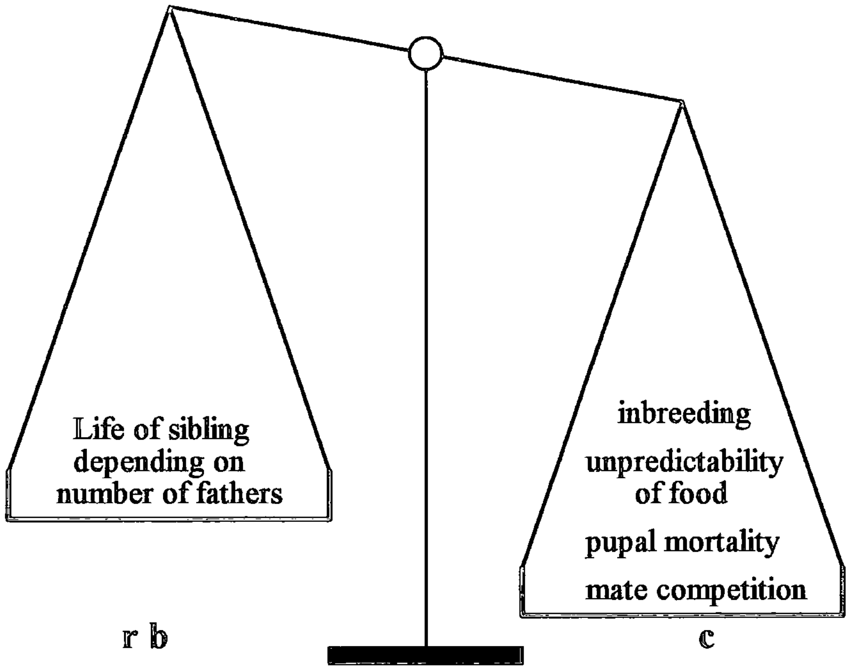
Between clutches, variation in cannibalism rates may be explainable by disparity in degree of genetic relatedness according to the number of fathers siring a clutch (including elevated relatedness through inbreeding) altering "r" in the equation $rb > c$. Although polyandry is usually suppressed by matrone, released from the male accessory gland, acting to make females refractory to further insemination, (Craig, 1967), genetic crosses using sex-linked alleles and employment of genetic markers have revealed cases of polyandry and multiple paternity in *A. aegypti*, *Anopheles* *gambiae* and several *Culex* species (Gwadz & Craig, 1970, Gomulski, 1990, Bullini, *et al.*, 1976, Kitzmiller & Laven, 1958). Multiple paternity could also be caused by sperm storage. For *T. digitatum* it has been revealed that multiple paternity and variation in the number of fathers can be determined by means of RAPD-PCR profiles. This technique could, in the future, also be applied to *T. moctezuma,* not only to determine within clutch relatedness but also to distinguish separate clutches.

Furthermore, if multiple insemination is rife, ramifications exist for attempts to control mosquito populations using irradiated males (but refer to Gomulski, 1990). At present time this would seem particularly applicable as visions of defeating malaria (the most prevalent insect-borne killer, causing 1-2 million deaths annually and likely to become more geographically wide-spread with global climate warming) chemically are fading, due to build-up of resistance, and the future is turning to genetically engineered forms that cannot carry the parasites or genetically introduced antibodies, (see Young, 1995).

- Biological factors such as the effect of moulting on vulnerability, (Fox, 1975c).

- Non-adaptive behaviour as a result of pathological conditions, for example small container size preventing assessment of prey.

Any of the above factors could outweigh costs of eating kin, as shown by figure 4.1 below, redress the cost-benefit trade-off of kin discrimination or consequent evolution of an inborn indiscriminative cannibalism trait (manifest in female Hyenas cubs, Frank, 1994), irrespective of environmental conditions. Personally, I would prioritise predictability of food supply and genetic fitness because obvious species-specific factors emphasise their roles as selective forces of cannibalism in *T. moctezuma.* Whilst further investigation, particularly into the biological basis, of sexual selection is required, even if variation in relatedness within a clutch exists, relatedness within will still be greater than relatedness among clutches and if larvae recognise kin larvae, why should they not recognise kin pupae?



Where costs and benefits relate to the donor (the larvae that avoids cannibalising kin).

Figure 4.1; Possible ecological factors outweighing benefits to inclusive fitness of kin discrimination in *T. moctezuma.*

An attempt was made to test for kin discriminative cannibalism in natural populations of *A. aegypti*. Unfortunately in the area of study too few *Aedes* were attracted to lay in the pots provided. In future more extensive sampling in a larger conurbation would be required. The results of this experiment would be augmentative to this kin selection study for several reasons. Variation in diapause cues, repeated oviposition and instalment hatching together may make it either more probable that large and small kin are in frequent contact or, alternatively, less probable, if instalment hatching does act to insure that potential predators and prey do not coexist. As detritivores, larvae will have a more predictable food source than *T. moctezuma*. Also, even if refractory hatching prevents coexistence, at the end of the rainy season, when shortage of time for development increases the cost of postponing hatching, hatching of small kin in the presence of large larvae may be expected to be more frequent.

Relative preference for cannibalism versus predation and occurrence of facultative cannibalism are other relevant aspects, to kin selection studies in general. It is highly unlikely that kin discrimination will occur if no discrimination at the species level is apparent. A prefatory experiment of six day-old *T. moctezuma*, preference for first instar conspecifics versus *C. mollis* first instars revealed a greater, but not statistically significant, predilection for heterospecifics, (pers. obs.).

4.4 Implications for and impacts of ovipositional behaviour

Findings of cannibalism experiments could aid explanation of the ovipositional behaviour of mosquitoes. Avoidance of conspecifics would be expected to be correlated to degree of cannibalism. Therefore *T. moctezuma* would be expected to evade conspecifics the most. More specifically, occurrence of kin discrimination in *T. moctezuma* would effect whether the optimal strategy would be to lay a few eggs in many pots or to concentrate resources on many lottery tickets for the same draw. Equally, turning the tables around, degree of aggregative oviposition can clearly contribute to explanation of level of kin discrimination.

Although chemical and abiotic aspects of ovipositional cues have been well documented, (for examples see Bentley & Day, 1989), little work has been completed regarding biotic cues such as the role of conspecifics (all life stages), other mosquito species and other tree-hole inhabitants. Perceptible exceptions include Blaustein & Kotler, 1993 who found that food and predation influenced oviposition choices in *Culiseta longiareolata* and Sherratt & Church, 1994, who found that *T. digitatum* prefers food rich sites, lays aggregatively but avoids large conspecific instars.

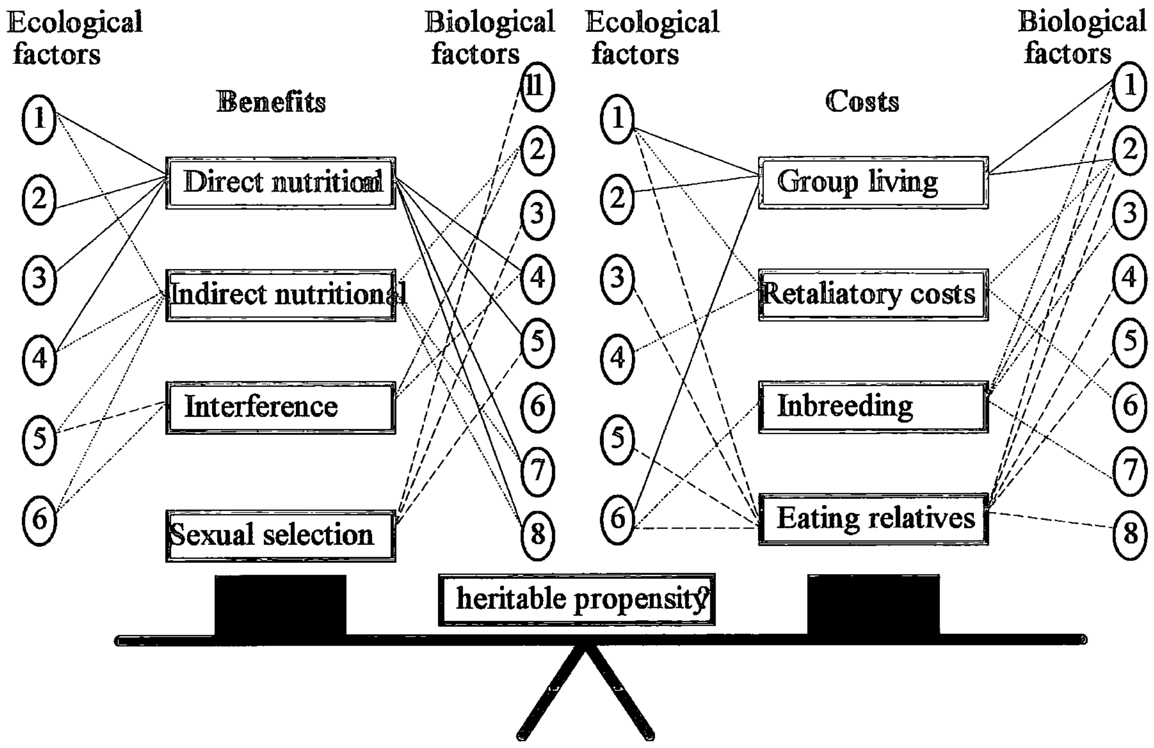
Optimality theory would predict that females should choose oviposition sites which maximise growth and survival of young and produce clutch sizes that optimise parental reproductive success. Both these strategies will have direct implications for natural occurrence of cannibalism as they will govern densities, age structures and species present. Conflicting currencies both within and between generations will compromise the degree to which optimality in any one can be achieved. Parent-offspring conflict can be expected as relatedness varies. To a parent all offspring co-inhabiting a tree-hole share the same number of genes but a single offspring will always be more related to itself. Within a generation multiple currencies enforce trade-offs. For example if predation or risks and consequences of inbreeding are larger threats than cannibalism to parental reproductive success (offspring development), it may be expected that ovipositional preferences will act to minimise these rather than cannibalism threats. In the context of these experiments, it could be expected that *T. digitatum* should avoid *T. moctezuma* more than conspecifics during oviposition. Investigation of actual ovipositional behaviours can allow inferences to be made about the relative significance of these determinants.

Practical tractability of inter-species ovipositional experiments poses a constraint to testing preferences, as revealed by an attempt to discern the effect of presence of eggs of *T. digitatum*, *C. mollis* and *T. moctezuma* to oviposition decisions. Due to species-specific peak ovipositional conditions and attractants (O'Malley, 1986) not only is simultaneous availability of eggs of all species problematic but, to collect an adequate number of ovipositional decisions, requires a long sampling period.

With respect to interactions between commonly coexisting mosquito species, relative threats to progeny laid in the presence of other eggs can be assessed by quantifying predatory and cannibalistic mortalities when equal-aged larvae are placed together. Preliminary study of three day-old larvae of *T. moctezuma, T. digitatum* and *C. mollis* suggest that *T. moctezuma* sustains the least predatory mortality and *C. mollis*, the most, (means of 0.3 and 2.4 larvae over 24 hours) with both *T. moctezuma* and *T. digitatum* preferring *C. mollis* to *T. digitatum* prey (2.4 as opposed to 0.3 and 1.6 as opposed to 0 mean number of prey eaten over 24 hours). Are these relative risks reflected in ovipositional decisions? Does *C. mollis* avoid laying in the presence of the other two species whilst *T. moctezuma* and *T. digitatum* track food sources in oviposition? If not, then why not?

4.5 Conclusive evolutionary significance of cannibalism

Experimentation of these neotropical species have shown that dynamics of cannibalism does indeed vary in accordance with ecological conditions such as food and density, advocating an adaptive significance. However by concentrating on modulating the relative advantage or disadvantage of one aspect, for example varying nutritional deficits or comparing between species expected to vary in benefits derived from kin discrimination, is unlikely to provide a comprehensive explanation for variability in cannibalism as displayed by lack of variation in kin discrimination amongst study species and fitness consequences in *A. aegypti* and *T. digitatum.* This is a consequence of two levels of compromise. Firstly a trade-off between all costs and benefits of cannibalism will occur, with cannibalism perceived as a strategy, expected to occur only in the ecological and biological conditions when benefits outweigh costs. A possible scenario of costs and benefits, together with their respective governing factors is shown by figure 4.2.



Numbers represent the following factors;

Ecological factors:

1. Predictability of food
2. Predation pressure
3. Ephemerality (and seasonal time)
4. Availability of alternative food
5. Dispersal conditions
6. Distribution of resources

Biological factors:

1. Type of mating (monogamy - promiscuity)
2. Oviposition - scattered vs. aggregative and simultaneous vs. instalment hatching
3. Mating behaviour, on the spot vs. dispersive
4. Method and range of feeding
5. Sex
6. Rate of growth, life history strategies
7. Commonness of species
8. Tolerance of desiccation and hunger

Figure 4.2; Plausible balance of costs and benefits of cannibalism in tree-hole mosquitoes together with ecological and biological factors governing their relative weights.

Secondly, to achieve the goal of life-time reproductive success, the cannibalistic strategy must fit into a framework with other potentially conflicting strategies such as mating success, predator avoidance etc. which can act to enhance or constrain cannibalism, irrespective of its overall fitness implications. An example of likely conflicts in

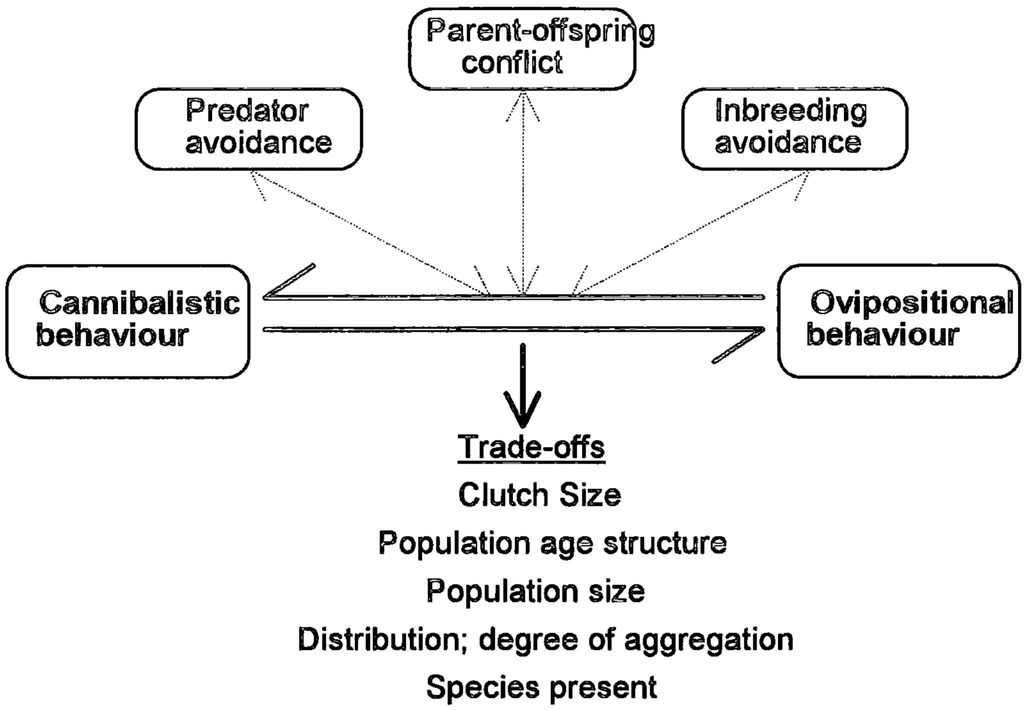


Figure 4.3; Hypothetical example of conflicting currencies to, and trade-offs between, cannibalistic and ovipositional behaviours

Focusing on individual costs and benefits, (as has been done in this study) and degree of correlation between observed variance with modification of single or several ecological factors is an effective, testable means to discriminate the factors important to costs and benefits, such as those hypothesised to explain exposed extreme variation in cannibalistic tendencies among co-inhabiting tree-hole species, lack of kin discrimination in *T. moctezuma* and fitness consequence in *A. aegypti* and *T. digitatum*, their relative importance and interactions with other behavioural strategies.

4.6 Implication for population dynamics and control

Besides evolutionary significance on an individual level, ramifications of cannibalism extend to population and community levels. The importance of mosquitoes as vectors for disease create more stringent control requirements as populations generally must be eradicated as opposed to only suppressed. There are a number of reasons why cannibalism will not be profitable and could possibly have detrimental effects despite its' significance as a key mortality factor. These concern consequences of cannibalism to population size and persistence;

- Population size;

1. "Soft deaths";

If most selective mortality through cannibalism replaces non-selective background deaths as a result of excess fecundity, little difference will be perceived in population size. Such a role can be envisaged for example in *Anopheles gambiae* giles as population studies reveal that first and second instars (those most vulnerable to cannibalism) represent key mortality stages (Aniedu *et al.*, 1993) and in Kestrels where the weakest and smallest fledglings were cannibalised (Bortolotti *et al.*, 1992). As many tree-hole mosquito larvae occupy ephemeral habitats, they have typically r-strategist life-histories, with most mortality occurring on young stages (Stubbs, 1977), so, with the possible exception of more starvation and desiccation tolerant species such as *Toxorhynchites* species, this rule can probably be applied liberally to tree-hole species. Cannibalism could act to increase rates of evolution if these selective deaths replace a more random process of elimination.

2. Adult fitness;

If, as suggested previously, cannibalism acts like dominance or spacing behaviour, by reducing numbers before starvation, consequenting larger more fecund adults with a greater longevity, will emerge. In fact, increased longevity in females could have severe, negative consequences for the disease vector potential of mosquitoes exhibiting renewed sexual receptivity after gonotrophic activity. Not only will a positive correlation exist between number of hosts infected and adult longevity, as blood meals are usually separated by oviposition periods of, for example, eight days in *A. sierrensis*, (Hawley, 1985), but also it will give increase probability of completion of vector incubation. As an example, *Annopheles* mosquitoes infected by the malaria-causing protozoans of the genus; *Plasmodium* can not re-infect for 10-15 days as, once ingested into the mid-gut, the protozoan must form an oocyte, mature and release sporozoites which migrate to the salivary glands (Burgess & Cowan, 1993).

However, in the light of this study, the role of physical interference at high larval densities, exhibited by the filter feeding *A. aegypti*, to inhibiting cannibalism, questions the density dependent nature of cannibalism and the above connotation. Research into the generality of this density interaction across species would be constructive. Rates of cannibalism in the *T. digitatum* kin discrimination experiment displayed no comparable difference in rates before and after the first pupation event, (Wilcoxon z value of -0.2395, p=0.8107). However, varying size, activity, feeding methods and conditions together make it probable that perceived densities varied. It would also be worth considering whether, in nature, cannibalism and instalment hatching in *A. aegypti* prevents increase to the inhibitory, high density levels, artificially set, in this study.

3. Resource utilisation;

If cannibalism allows utilisation of two separate resource bases (where prey use a resource inaccessible to potential cannibals), population carrying capacities can be increased in a given habitat, (see Ladle, 1991, for pike). Small size and limited complexity of tree-hole habitats curtails opportunity for such separation

- Population persistence and stability;

The consequences of cannibalism to population dynamics, whether it has a stabilising effect, promotes oscillations in numbers or allows persistence of populations in stressful conditions, such as providing a lifeboat strategy for increased persistence in fluctuating food conditions (possible support derives from increased cannibalism rates and expression in *A. aegypti* and in conditions of food scarcity in *T. digitatum,* Church and Sherratt, submitted) will clearly have implications to the damage potential, tenacity and control of pest populations.

Where oscillations are promoted, often with the complete predation of year-classes (Vanbuskirk, 1992), particularly applicable to cases of size-dependent cannibalism (Hastings & Costantino, 1991), such as cohort formation in *T. digitatum,* measures to control those classes that would be cannibalised anyway would be uneconomical. Where chemicals are used, selective applications could prolong build up of resistance, which is frequently found to be correlated to contact with pesticides. Non-size-dependent cannibalism, exhibited in *T. moctezuma* could be expected to act more like territorial behaviour, dampening oscillations and increasing stability of populations. The multivoltine nature and short generation time of tropical mosquitoes permits feasible refutation of this proposed dichotomy in population stability consequences. Efficiency of control measures are likely to vary in accordance with such factors.

Cannibalism is unlikely to cause extinction as its expression is often a function of potential predator : prey density, cannibals can switch to other food sources in scarcity, prey can survive in partial refuges, the often size-dependent nature ensures that it is a temporally short-term phenomena and persistence of populations selects against excessive cannibalism.

Several attempts have been made in the past to use *T. moctezuma* as a biological control agent of *A. aegypti*, some of which have been successful. However utilisation of *Toxorhynchites* can benefit from knowledge of their cannibalistic behaviours. For example, there would be little point to placing many control larvae in one pot if they cannibalise each other first or if kin are more likely to coexist they would be more effective in abolishing *A. aegypti* populations.

- Community structure;

On a community level cannibalism can be expected to have implications that have, as yet, been largely unexplored. Pertinent questions include implications of cannibalism to species coexistence; would extreme intra-specific competition relax inter-specific competition and allow more closely related species to coexist? and consequences to trophic levels further up food chains, for example to the available food supply and population dynamics of predators of cannibalistic species.

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Experiment 2, T. digitatum																					
clutch	sex	rep.	Daily number of first instars consumed										Adult	emergence		Controls;			Adult	emergence	
			day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	tibia size	time		clutch	sex	rep	tibia size	time	
9	1	2	0	0	2	2	2	0	0	0			20.5	12.5		9	2	3	21	14	
9	1	3	1	0	0	1	1	0	1	1	0		19.5	13.5		9	2	4	20.5	15	
9	1	4	0	0	1	3	0	1	0	2	0	1	21.5	13		9	2	7	21	14	
9	1	5	1	0	2	0	2	0	0				22	14.5		9	2	8	21	14.5	
9	1	8	0	1	3	1	1	1	0	1			21	14.5		10	1	1	19	16	
9	2	1	0	1	3	3	1	1	0	0			20	12.5		10	1	2	21	14	
9	2	6	0	0	1	2	2	1	1	0	0		20	14.5		10	1	3	19.5	12.5	
9		7	0	0	2	2	2	1	0	1			20.5	13.5		10	1	4	21	14.5	
10	1	2	0	0	0	0	2	0	0	0	0		19.5			10	1	5	19	16	
10	1	3	1	0	1	1	1	2	0				21	14		10	1	6	19.5	12.5	
10	1	4	0	0	2	1	2	2	0	1	1		21.5	14		10	1	8	21	13.5	
10	1	5	0	1	1	1	0	3	1	1	0		20.5	16		10	2	7	22.5	14.5	
10	1	7	1	0	0	0	3	0	1	0	0			11		11	1	1	19.5	12.5	
10	2	1	0	0	0	0	1	2	1	0	0		21	12.5		11	1	2	20	12	
10	2	6	0	0	3	1	2	1	1	0	0			13.5		11	1	4	20	11	
10	2	8	1	1	2	2	1	0	2	1	0	1	20	13.5		11	1	5	20	13.5	
11	1	1	0	0	1	0	1	3					20	13.5		11	1	7	18.5	12	
11	1	5	0	1	1	2	2	1	0				22	14		11	2	3	19.5	13.5	
11	1	7	0	0	1	1	1	1	0	1			20	14.5		11	2	6	20.5	14	
11	2	2	1	0	1	3	1	0	0	1	0		21	13.5		11	2	8	20.5	14	
11	2	3	0	1	0	2	1	1	2	0			19	12.5		12	1	2	20	12.5	
11	2	4	0	0	3	1	0	1	0	1	0		19	12.5		12	1	5	19	12.5	
11	2	6	1	0	1	2	1	1	1	1	0		20.5	13		12	1	6	19.5	13.5	
11	2	8	0	3	1	3	2	1	0	0			19.5	13.5		12	1	7	19	12	
12	1	1	0	0	1	1	0	1	0	0				13		12	1	8	21	15	
12	1	2	0	2	2	3	1	2	0				19.5	14		12	2	1	18.5	16.5	
12	1	3	0	1	3	2	1	1	0	0			20.5	14		12	2	3	20	13.5	
12	1	4	0	0	1	1	1	2	1	1			20	15		12	2	4	20	14.5	
12	1	6	0	0	0	2	2	0	0	0			19.5	14.5		13	1	3	18	13.5	
12	1	7	0	0	1	0	3	0	1	0	0		19.5	14.5		13	1	5	19	14	
12	1	8	0	0	2	3	2	3	2	1	0		21	14		13	1	7	20.5	14	
12	2	5	0	1	1	3	0	0	1	1	0	0	19	13.5		13	2	1	20	16	
13	1	2	1	1	0	3	2	2	0	0	0		19	12.5		13	2	2	20.5	14	
13	1	3	0	0	0	0	2	2	1	0	1		19	14		13	2	4	21.5	13.5	
13	1	4	1	1	0	2	0	2	2	0	0		19	14.5		13	2	6	19	16.5	
13	1	5	0	2	1	1	3	2	2	0			20	14.5		13	2	8	19	12.5	
13	1	6	0	2	0	0	0	1	1				20	12		14	1	1	20	14	
13	1	8	0	0	1	1	2	1	0	1	0		20	13.5		14	1	2	20	12	
13	2	1	0	0	1	2	2	3	3	1	0			13		14	1	7	19.5	12	
13	2	7	0	0	1	2	2	1	1	0	0		20	12.5		14	2	3	20	15	
14	1	1	0	0	2	1	0	1	1				19.5	13.5		14	2	4	22	16.5	
14	1	2	0	1	2	2	2	0	0	1			21	14		14	2	5	19.5	16	
14	1	4	0	0	0	0	0	0	0	1			20.5	14.5		14	2	6	22	14.5	
14	1	6	0	0	2	1	0	0	0				20	13.5		14	2	8	21	15	
14	1	7	0	0	1	2	1	0	3	0			15.5	14.5		15	1	2	19	13.5	
14	1	8	0	1	0	2	3	1	0	0	0		20	14.5		15	1	3	18	12.5	
14	2	5	0	0	1	3	1	0	1	0	0		19	14		15	1	4	21	13.5	
14		3	1	0	1	3	1	2	1	0	0	1	19.5	14		15	1	6	20.5	14	
15	1	2	0	0	2	2	2	1	0	1			21	14		15	1	8	20	13	
15	1	3	0	1	1	1	2	2	1	2	1		20	15		15	2	1	19.5	16	
15	1	7	0	0	0	2	1	1	0	0	1		19.5	11		15	2	5	20	15	
15	1	8	1	0	1	1	2	0	1	2	0		20	13		15	2	7	21	14.5	
15	2	4	1	2	3	0	1	0	1	1	0		20.5	13		16	1	3	18.5	13	
15	2	5	0	0	1	1	3	2	2	0	1		21	13.5		16	1	4	20	12.5	
15	2	6	0	1	0	0	3	0	0	2	0	0	21	14		16	1	7	19	12.5	
15		1	1	1	0	1	1	1	1	0	0		19.5	13		16	1	8	18	12.5	
16	1	1	0	1	1	3	1	3					21	14		16	2	2	21.5	12	
16	1	2	0	0	1	1	2	0	1	1						16	2	5	21.5	13.5	
16	1	8	0	0	2	2	3	1	0	0						16	2	6	21.5	16	

Experiment 2. T. digitatum																				
clutch	sex	rep.	Daily number of first instars consumed										Adult	emergence		Controls;			Adult	emergence
			day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	tibia size	time		clutch	sex	rep	tibia size	time
1	1	4	0	1	1	2	2	2	1	1	0			14.5		1	1	2	20.5	16
1	1	6	0	0	1	0	1	0	2	0	1		21	14.5		1	1	8	21	13.5
1	1	7	0	0	1	1	2	0	1	2	0		19.5	14		1	2	3	22.5	14
1	2	1	1	0	0	1	3	1	1	1	0		22	14		1	2	4	22	13.5
1	2	2	0	0	0	3	2	2	1	1	0	0	21	15		1	2	5	21	14.5
1	2	3	0	1	2	2	1	0	0	0			21	13.5		1	2	6	21	16
1	2	5	0	1	1	2	2	0	0	1	0		22	14		1	2	7	21	12.5
1	2	8	1	0	1	0	3	3	0	0	0	0	22	15		2	1	1	20.5	14
2	1	2	0	0	2	3	2	2	1	1	1		19.5	14		2	1	2	20	14
2	1	3	0	1	1	1	2	2	2	1			20	13		2	1	4	20	14
2	1	4	0	0	2	1	3	2	1	0	0		20.5	14		2	1	7	21	14.5
2	1	5	0	1	2	0	0	1	1	0	0		20.5	14		2	1	8	19	15
2	1	6	0	0	0	2	2	0	1	1	0			14		2	2	3	21	14.5
2	1	7	1	2	1	1	1	1	1	0	0		21	14		2	2	5	20.5	14.5
2	1	8	0	0	0	2	2	1	1	0	0	0	21	15		2	2	6	20	16.5
2	2	1	2	0	1	1	2	1	1	0	0	1				3	1	1	21	14.5
3	1	3	0	0	1	3	1	3	2	1	0		20	14.5		3	1	3	19.5	11
3	1	4	0	0	1	1	2	2	0	1				13.5		3	1	6	19.5	13.5
3	1	7	0	1	3	1	2	2	2	0			21	13.5		3	1	8	19.5	14
3	2	1	0	0	0	3	2	3	0				21	12.5		3	2	2	20.5	16
3	2	2	1	0	1	1	2	1	0	1	1		21	14.5		3	2	4	21	16
3	2	5	0	2	1	1	0	0	1	1			22	13.5		3	2	5	22	14.5
3	2	6	0	1	0	2	2	1	1	0	0	1	20	16		3	2	7	21	15
3	2	8	1	0	1	2	2	3	0	1	0	1	22	15		4	1	1	20.5	12.5
4	1	2	0	0	1	2	0	2	1					12.5		4	1	4	21	14.5
4	1	7	0	0	1	0	2	3	1	0	2		20	14.5		4	1	6	18.5	15
4	2	1	0	1	1	0	2	3	0	0			20.5	13.5		4	1	8	19	12.5
4	2	4	0	1	1	2	2	2	2	0	1		22	14		4	2	2	21	14.5
4	2	5	0	1	1	2	0	0	0	3	0		21.5	14		4	2	5	20	16
4	2	6	1	0	1	1	1	0	1	1	0		20.5	14.5		4	2	7	20	16
4	2	8	1	1	0	3	1	2	0	0			20.5	13.5		5	1	1	20	14.5
4		3	1	1	1	3	2	1	1	1	0	0	19	12		5	1	5	20	12.5
5	1	3	0	2	0	1	2	0	0					11		5	1	6	19.5	12.5
5	1	5	1	0	0	1	1	1	0	1	0	0	20.5	14.5		5	2	2	19	12
5	1	6	0	1	2	1	0	0					21	14		5	2	3	20	14.5
5	2	1	0	0	0	0	0	2	1	0	0		20	14		5	2	4	21	14.5
5	2	2	0	0	2	2	2	1	1	2	0		21	15		5	2	7	20	16
5	2	4	0	0	1	3	1	2	1	0	2		21	14		5	2	8	20	14.5
5	2	7	1	0	0	1	2	0	0	0	0	1	22	14		6	1	1	19.5	14.5
5	2	8	0	0	1	0	0	0	2	1	0		20.5	14		6	1	5	20	14
6	1	2	0	0	1	2	2	1	0	1	1		18.5	15		6	1	6	18.5	13.5
6	1	4	0	0	0	1	0	1	1	0	1	0	19.5	14.5		6	1	8	20.5	12.5
6	1	5	0	0	0	1	3	0	1	3	0		21	14		6	2	2	21	12.5
6	2	1	0	0	2	2	0	2	1	0	0		20	14		6	2	4	21	14
6	2	6	0	1	0	1	2	0	1	0	0		20	16		6	2	7	19.5	15
6	2	7	0	0	1	1	0	0	1	1	1		20	13		7	1	7	20	13
6	2	8	0	1	2	0	1	0	0	0			19	12		7	1	8	20	12.5
6		3	0	0	0	2	1	0	1	1	1	0	19.5	13.5		7	2	1	21	14
7	1	1	0	0	3	1	2	2	0							7	2	2	21	11
7	1	4	0	0	1	0	2	3	1	0			21.5	12.5		7	2	3	19	15
7	1	5	0	0	2	3	1	2	0	0			21	13.5		7	2	5	22	14.5
7	1	6	0	0	1	2	3	0	1				21.5	15		7	2	6	20	14.5
7	1	7	1	0	1	1	1	2	0	0			21	14.5		8	1	1	20	13.5
7	2	2	0	0	3	1	1	2	3	1	1	0	22	13.5		8	1	3	20	12
7	2	3	0	0	1	2	1	2	2	0	1		20.5	13		8	1	5	20.5	11
7	2	8	0	1	1	1	3	1	2	0			20	12.5		8	1	6	20.5	12.5
8	1	2	0	0	1	2	2	2	0	0			20.5	13.5		8	2	2	22	13.5
8	1	3	1	1	0	1	2	0	0				21	13		8	2	4	21	14.5
8	1	6	0	1	1	2	0	1	0	0			22	13.5		8	2	7	23	14.5
8	1	7	0	0	3	0	2	1	0	0			22	15		8	2	8	22	12.5
8	2	1	0	0	1	1	0	2	0	0			21	12.5		9	1	2	22	15
8	2	4	0	1	2	1	3	3	2	2	1	0	20.5	13.5		9	1	5	19.5	13
8	2	8	1	1	0	1	3	1	0					14		9	1	6	20	14.5
8		5	0	2	2	2	2	2	1	0	2	0	19.5	15		9	2	1	20	13.5

Experiment 3, C. mollis; Cells represent daily number of small instars absent in kin and non-kin situations

e denotes final pupation

Clutch	Day 1 kin	Day 1 non	Day 2 kin	Day 2 non	Day 3 kin	Day 3 non	Day 4 kin	Day 4 non	Day 5 kin	Day 5 non	non totals	% male	% female	kin totals	% male	% female
1	0	0	0	0	0	0	0	0	e	e	0	0.5	0.5	0	0.75	0
2	0	0	0	0	0	0	0	e	e	e	0	0.75	0.25	0	0.5	0.5
3	0	0	1	0	0	0	e	0	e		0	0.75	0.25	1	0.5	0
4	0	0	0	0	0	0	0	0	.	e	0	0	0.75	0	0.5	0.25
5	0	0	0	0	0	0	0	0	e	e	0	0.25	0.25	0	0.25	0
6	0	0	0	0	0	0	0	0	e	e	0	0.25	0.5	0	0.25	0
7	0	0	1	0	0	0	e	0	e		1	0.25	0.75	1	0.25	0.5
8	0	0	0	0	0	0	e	0	.		0	0.5	0.25	0	0.5	0.5
9	0	0	1	0	0	0	0	0	.	e	0	0.5	0.25	1	0.75	0.25
10	0	0	0	0	0	1	0	0	e	e	1	0.25	0	0	0.75	0.25
11	0	0	0	2	e	e	.	.	e		2	0	1	0	0.5	0.5
12	0	0	0	0	0	e	e	.	.		0	0	1	0	0	0.75
13	0	0	3	0	4	e	e	.	.		0	0.75	0	7	0	1
14	0	0	0	0	0	0	e	e	.		0	0.25	0.75	0	0.25	0
15	1	0	1	0	e	e		.	.		0	0	0.75	2	0	1
16	0	0	0	0	e	0	.	e	.		0	0.25	0.75	0	0.25	0.75
17	1	0	0	0	e	e	.	.	.		0	0.25	0.75	1	0.25	0.75
18	0	0	0	0	e	e	.	.	.		0	0.25	0.75	0	0.75	0.25
19	0	0	0	0	0	0	e	e	.		0	0.25	0.5	0	0.25	0.75
20	0	0	0	0	0	e	e	.	.		0	0.5	0.5	0	0.75	0.25
21	0	0	0	0	e	e	.	.	.		0	0.25	0.5	0	0.5	0.5
22	0	0	0	0	0	e	e	.	.		0	0.25	0.75	0	0.5	0.5
23	0	0	0	0	e	e	.	.	.		0	0	0.75	0	0	1
24	0	0	0	0	e	e	.	.	.		0	0.5	0.5	0	0	0
25	0	0	0	0	e	e	.	.	.		0	0.5	0.5	0	0.25	0.75
26	0	0	2	0	e	e	.	.	.		0	0.5	0.5	2	0	0.25
27	0	0	0	0	0	0	e	e	.		0	0.5	0	0	0.25	0.75
28	0	0	0	0	e	0	.	.	.		0	0.5	0.5	0	0.25	0.75
29	0	0	0	0	0	0	.	0	.	e	0	0	0.25	0	0	0
30	0	0	0	0	0	0	.	0	.	e	0	0	1	0	0.25	0.75

Experiment 3, T. digitatum; Number of small eaten daily in kin and non-kin situations									**e denotes all cannibals pupated**															
Clutch	Day 1 kin	Day 1 non	Day 2 kin	Day 2 non	Day 3 kin	Day 3 non	Day 4 kin	Day 4 non	Day 5 kin	Day 5 non	Day 6 kin	Day 6 non	Day 7 kin	Day 7 non	Day 8 kin	Day 8 non	Day 9 kin	Day 9 non	Total kin	% males	% females	Total non	% males	% females
1	1	3	0	2	1	0	0	0	1	1	1	0	e	0		e			4	1.00	.00	6	.50	.50
2	0	0	0	1	0	0	1	0	0	0	0	3	3	0	3	0	0	2	7	.00	1.00	7	.25	.75
3	3	4	5	1	0	0	0	0	0	0	0	0	e	e					8	.25	.25	5	.75	.25
4	1	0	1	1	0	1	1	1	2	1	0	1	1	0	0	3	1	2	7	.50	.50	11	.25	.75
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	0	1	0	2	3	1	0	0	0	3	2	0	0	1	1	0	4	e	10	.50	.50	7	.50	.50
7	2	0	0	2	0	0	0	1	0	0	1	0	0	e	2		e		5	.75	.25	2	1.00	.00
8	0	1	0	1	0	0	1	1	0	0	0	0	1	0	2	0	e	e	4	.50	.50	3	.50	.25
9	1	2	0	0	2	0	2	0	2	0	2	1	0	0	0	0	e	e	9	.25	.75	3	.25	.50
10	1	0	0	0	0	0	0	1	0	6	0	2	e	1		0		e	1	.50	.00	11	.00	.50
11	3	2	1	0	1	0	0	0	2	0	0	1	0	2	e	e			6	.25	.75	5	.50	.25
12	0	2	1	0	1	0	0	0	0	0	0	0	0	0	e	e			2	.25	.25	2	.50	.25
13	1	2	0	0	1	3	0	0	0	0	0	1	0	0	e	e			2	.25	.50	6	.50	.25
14	1	0	0	0	0	2	2	0	0	0	0	0	3	0	e	e			6	.75	.00	2	.00	.00
15	3	5	0	2	0	0	0	0	2	0	1	2	2	0	1	e	e		9	.75	.25	9	.50	.50
16	2	0	3	2	1	1	1	0	0	1	0	0	0	0	1	e	e		8	.25	.75	3	.50	.50
17	2	2	0	0	2	1	1	1	0	0	1	0	0	0	0	0	0	e	6	.25	.50	4	.50	.50
18	0	2	1	1	0	0	0	1	1	0	0	0	2	1	e	0		e	4	.50	.25	5	.25	.50
19	1	0	0	2	0	0	0	0	0	0	1	0	0	0	e	e			2	.50	.50	4	.50	.25
20	0	1	1	0	0	0	0	0	2	0	1	0	1	0	0	0	e	e	6	.75	.00	1	.50	.25
21	3	2	0	1	2	0	0	0	2	0	2	0	2	0	0	0	e	e	11	.25	.25	3	.00	.25
22	3	2	2	2	1	1	0	0	0	0	0	0	1	4	e	0		e	7	.50	.50	9	.25	.50
23	5	5	0	2	0	0	0	1	0	1	1	1	e	0		0		e	6	.00	.50	10	.50	.25
24	2	1	1	4	0	2	0	0	2	0	1	0	e	e					6	.75	.25	7	.25	.50
25	4	2	1	1	0	1	2	0	0	0	0	0	1	0	1	1	e	e	9	.50	.50	5	.75	.25
26	6	7	0	-	0	-	1	-	0	-	0	-	e	-		-		-	7	.50	.50	-	-	-
27	1	0	2	1	2	0	0	0	0	0	0	0	e	1		0		e	5	1.00	.00	2	.50	.00
28	2	3	1	2	1	4	1	1	0	0	0	0	e	e					5	.25	.75	10	.00	.25
29	1	1	1	1	0	0	1	0	0	0	0	0	e	e					1	.50	.25	2	.50	0.5
30	2	3	1	0	0	1	0	0	0	2	0	0	e	e					3	0.25	0.75	6	0	0.25