

# Testing for context-dependence in a processing chain interaction among detritus-feeding aquatic insects

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**Abstract.** 1. Scirtid beetles may benefit mosquitoes *Ochlerotatus triseriatus* (Say) by consuming whole leaves and leaving behind fine particles required by mosquito larvae. Such interactions based on the sequential use of a resource that occurs in multiple forms are known as processing chains.

2. Models of processing chains predict that interactions can vary from commensal (0, +) to amensal (0, -), depending on how quickly resource is processed in the absence of consumers.

3. The scirtid-*O. triseriatus* system was used to test the prediction derived from processing chain models that, as consumer-independent processing increases, scirtids benefit mosquitoes less. Consumer-independent processing rate was manipulated by using different leaf species that vary in decay rate, or by physically crushing a single leaf type to different degrees.

4. Although scirtids increased the production of fine particles, the effects of scirtids on mosquitoes were weak and were not dependent on consumer-independent processing rate.

5. In the leaf manipulation experiment, a correlation between scirtid feeding and consumer-independent processing was detected. Numerical simulations suggest that such a correlation may eliminate shifts from commensal to amensal at equilibrium; because mosquito populations are typically not at equilibrium, however, this correlation may not be important.

6. There was evidence that mosquitoes affected scirtids negatively, which is inconsistent with the structure of processing chain interactions in models. Processing chain models need to incorporate more detail on the biology of scirtids and *O. triseriatus*, especially alternative mechanisms of interaction, if they are to describe scirtid-*O. triseriatus* dynamics accurately.

**Key words.** Amensal, commensal, context-dependent interaction, leaf processing, *Ochlerotatus triseriatus*, scirtid beetle, tree hole.

## Introduction

Recent models of species interactions have emphasised the dependence of the nature of ecological interactions on the environment in which they occur (Addicott, 1981; Wolin & Lawlor, 1984; Hernandez, 1998). Such context-dependent outcomes have been demonstrated in many field studies,

covering a breadth of systems (e.g. Cushman & Whitham, 1989; Setälä *et al.*, 1997; Zamora, 1999).

One type of species interaction predicted to show context-dependence is a processing chain (Heard, 1994a,b, 1995). Processing chains are based on the sequential use of one resource that occurs in multiple states, with multiple consumer species specialising on different resource states. The incomplete use and subsequent alteration of the resource by initial (upstream) consumers affect the availability or value of the resource to subsequent (downstream) consumers. Due to the sequential use of resources, processing chains are necessarily unidirectional (i.e. the initial consumer affects subsequent consumers but not vice versa). Therefore,

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processing chain interactions may fall somewhere along a continuum from beneficial to detrimental for the downstream consumer, depending on the specific characteristics of the environment and interacting species (Heard, 1994a, 1995).

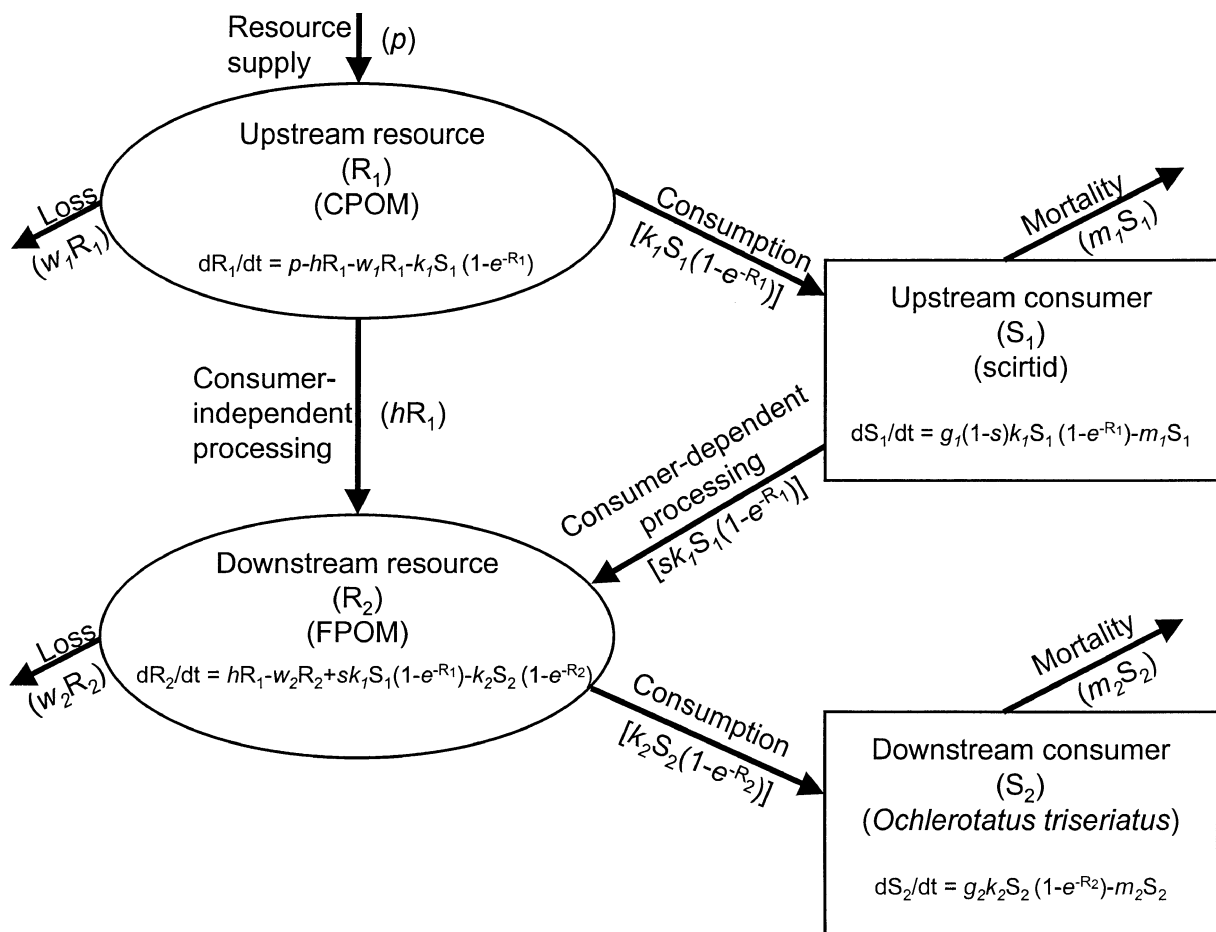
Heard's (1994a, 1995) analysis of a processing chain model (Fig. 1) gives specific predictions about the ecological contexts favouring commensal (0, +) and amensal (0, -) outcomes. Heard (1994a) showed that three parameters are especially important determinants of the nature of the interaction at equilibrium: loss rate of the unprocessed (upstream) resource (upstream loss rate,  $w_1$ ), the rate at which the upstream resource is converted to the processed (downstream) state in the absence of consumer species (consumer-independent processing rate,  $h$ ), and inefficiency in feeding by the upstream consumer (sloppiness,  $s$ ). Sloppy upstream consumers increase the production of an

otherwise inadequate supply of downstream resource, thereby benefiting downstream consumers. This benefit occurs when:

$$s > h/(h + w_1) \quad (1)$$

Otherwise, if  $s < h/(h + w_1)$ , upstream consumers pre-empt the processing of resource that would otherwise be destined for downstream consumer consumption, resulting in an equilibrium amensal interaction (Heard, 1994a, 1995). Thus, this model predicts that upstream consumers benefit downstream consumers most at low consumer-independent processing rates and benefit them least (or even harm them) at high consumer-independent processing rates.

Characteristics of processing chains have been described in soil, phytotelm, carrion, dung, lake, grassland, and fermentation communities, involving many types of organism (Heard, 1994a). Systems involving decomposers and detri-



**Fig. 1.** Model of a two-species processing chain. Equations describe change in resource pools and consumer populations:  $p$  is the resource supply,  $R_1$  and  $R_2$  are the amount of upstream and downstream resource respectively,  $w_1$  and  $w_2$  are the loss rates of upstream and downstream resource respectively,  $h$  is the consumer-independent processing rate,  $S_1$  and  $S_2$  are the upstream and downstream consumer populations respectively,  $k_1$  and  $k_2$  are the maximum feeding rates of upstream and downstream consumers respectively,  $g_1$  and  $g_2$  are the growth rates of upstream and downstream consumers respectively,  $m_1$  and  $m_2$  are the mortality rates of upstream and downstream consumers respectively, and  $s$  is the sloppiness of the upstream consumer. CPOM and FPOM denote coarse particulate and fine particulate organic matter respectively. Adapted from Heard (1995).

tivores seem particularly likely to contain processing chains, probably due to their high frequency of sequential change in resource quality (Heard, 1994a). Processing chains are likely in the insect-dominated communities that occupy water-filled tree holes and artificial containers. Decomposing plant detritus is thought to be the most important resource for tree hole insects (Kitching, 1971; Fish & Carpenter, 1982; Carpenter, 1983). The type, quality, and amount of plant detritus have important consequences for members of these communities (Fish & Carpenter, 1982; Léonard & Juliano, 1995; Yanoviak, 1999).

Three groups of tree hole insect are especially interesting as possible members of processing chains: mosquitoes *Aedes* and *Ochlerotatus* spp., ceratopogonid midges *Culicoides* spp., and scirtid beetles *Helodes* and *Prionocyphon* spp. Larvae of *Aedes* and *Ochlerotatus* filter feed in the water column and brush container and leaf surfaces to collect fine particulate organic matter (micro-organisms and detritus particles) but cannot generally consume large pieces of detritus (coarse particulate organic matter) directly. Similarly, larval *Culicoides* are benthic deposit feeders on fine particulate organic matter. Larvae of tree hole scirtids scrape or shred leaves, however, leaving behind fine particulate organic matter in the form of degraded leaf matter and faeces (Barrera, 1996; Paradise & Dunson, 1997). Scirtid larvae appear to act as upstream facilitators of downstream consumer *Aedes*, *Ochlerotatus*, and *Culicoides* via processing chains. The presence of scirtid larvae results in faster development of *Ochlerotatus* (formerly *Aedes*) *geniculatis* (Olivier) in the field (Bradshaw & Holzapfel, 1992), and has been found to increase the growth of *Culicoides* (Paradise & Dunson, 1997) and *Ochlerotatus* (formerly *Aedes*) *triseriatus* (Say) in laboratory experiments (Paradise, 1999, 2000). Furthermore, scirtids have been found to increase the degradation of coarse particulate organic matter (Paradise & Dunson, 1997; Paradise, 1999, 2000) and scirtid faeces are a food resource for mosquito larvae (M. P. Daugherty, unpublished).

Although previous studies have identified characteristics of tree hole insects which suggest that processing chains may occur, no study has tested directly whether the dynamics of those species are described by processing chain models. If scirtids are the first step in a processing chain, they should facilitate or inhibit other container insects, depending on resource processing and loss rates. Paradise (1999, 2000) noted variable outcomes of interactions between scirtids and *O. triseriatus* depending on the amount of leaf litter present, however this result is not obviously consistent with predictions of processing chain models because resource input is not predicted to alter the nature of processing chain interactions. If scirtids and other container insects constitute a processing chain, scirtids should affect these other species via the production of necessary fine particulate organic matter (Heard & Richardson, 1995). Although scirtids increase the disappearance of coarse leaf litter (Paradise, 1999, 2000), a net increase in the production of fine particles has not been documented.

In the work reported here, the scirtid-*O. triseriatus* system was used to test the hypothesis that processing chain dynamics are governed by consumer-independent resource processing rates. Container mosquito populations in the temperate zone probably reach equilibrium rarely due to intervening freezing or drought (e.g. Bradshaw & Holzapfel, 1983, 1985, 1988). Heard's processing chain model (1994a), however, examined factors that control context-dependence at equilibrium; context-dependence in short-term dynamics was not documented explicitly. Therefore, as an initial step, it was verified that the conditions described in eqn 1 apply to short-term processing chain dynamics. Numerical simulations of downstream consumer dynamics under different sets of resource processing and loss rates were evaluated in the very short term as well as at equilibrium. Next, the prediction that outcomes of processing chains shift from beneficial to detrimental for downstream consumers at high consumer-independent processing rates (Heard, 1994a, 1995) was evaluated empirically in two experiments that manipulated consumer-independent processing rate in different ways. First, a source of natural variation in consumer-independent processing rate was used: differences in decay rate among leaf species (Fish & Carpenter, 1982; Yanoviak, 1999). Scirtids would be expected to benefit mosquitoes most in the presence of the leaf species that naturally decomposes slowly, however scirtid feeding preferences may weaken the context-dependence. In streams, leaf-shredding macro-invertebrates prefer leaf species that decay quickly (Kaushik & Hynes, 1971) and consume them at a greater rate (Bird & Kaushik, 1985), suggesting that consumer-independent processing rate and shredder feeding may be correlated positively. The consequences of such a correlation within a processing chain framework are not known. Therefore, correlation between leaf decay rate and scirtid feeding was tested for, and the consequences of such a correlation for consumer species dynamics were explored via numerical simulations. As a second test of the prediction that eliminates any such correlation, consumer-independent processing rate was manipulated by physically crushing varying amounts of a single leaf species into fine particles. In both experiments, the effect of scirtids on mosquitoes was determined at each consumer-independent processing rate, and two assumptions were evaluated: that scirtids increase the conversion of coarse particles to fine particles, and that interactions between these species are unidirectional (i.e. mosquitoes do not affect scirtids).

## Materials and methods

### *Numerical simulations: short-term dynamics and consequences of correlation*

The possibility that short-term processing chain dynamics may exhibit context-dependence was explored by comparing numerical simulations of a processing chain model (see Fig. 1; Heard, 1994a, 1995) during the very early

stages of population growth. Conditions were set so that manipulations of consumer-independent processing rate and upstream consumer presence would result in equilibrium context-dependence according to eqn 1 (e.g.  $s=0.4$ ,  $w_1=0.6$ ,  $h=0.05-0.15$ ). Equilibrium context-dependence was verified by comparing downstream consumer population sizes qualitatively after 500 time steps ( $t=500$ ) with and without upstream consumers present for a given level of consumer-independent processing rate, then comparing those differences among consumer-independent processing rates. Context-dependence during the short term ( $t=0-10$ ) was tested by qualitatively comparing differences in slopes of downstream consumer population growth with and without upstream consumers present, then comparing those differences among consumer-independent processing rates. Context-dependence could be manifest as either differences in the magnitude of benefit/harm that downstream consumers derive from upstream consumers being present at different consumer-independent processing rates (i.e. *quantitative* context-dependence; differences between slopes or equilibrium population sizes vary systematically with consumer-independent processing rate), or it could be manifest as an outright switch from upstream consumers benefiting downstream consumers to harming them, or vice versa [i.e. *qualitative* context-dependence; the sign (+/-) of differences in slopes or equilibrium population sizes changes with consumer-independent processing rate]. Several different permutations of these *types* of context-dependence were evaluated, which showed similar levels of context-dependence in the short term, but the focus was on the equilibrium commensal case for the sake of brevity and because scirtid-mosquito interactions are thought to be largely commensal.

The consequences of a positive correlation between consumer-independent and consumer-dependent processing on processing chain dynamics were explored by comparing the results of the first set of numerical simulations where upstream consumer feeding rate was held constant ( $k_1=0.38$ ) with simulations where upstream consumer feeding rate was correlated positively with consumer-independent processing rate. In this second set of simulations, upstream consumer feeding increased with consumer-independent processing rate [values used were  $(h, k_1) = (0.05, 0.30); (0.08, 0.38); (0.10, 0.44); (0.15, 0.48)$ ]. Pairs of downstream consumer growth curves were compared qualitatively at a given consumer-independent processing rate for those with and without upstream consumers present, then the differences were compared among the consumer-independent processing rates. The effects of the correlation were tested at equilibrium ( $t=500$ ) as well in the short term ( $t=0-10$ ).

#### Multiple leaf types

Consumer-independent processing rate was manipulated by using leaves from four tree species that form a series of decreasing decomposition rates: slippery elm *Ulmus fulva*,

sugar maple *Acer saccharum*, white oak *Quercus alba*, and sycamore *Plantanus occidentalis*. In July 1997, leaves were collected from several trees of each species, at several locations within a tree. Although senescent leaves accumulate more typically in tree holes, only fresh leaves were available due to the timing of the experiment. The leaf blades were washed, broken into 10–15 cm<sup>2</sup> pieces, and the pieces were dried for >3 days at 60 °C. Then 0.3333 g (measured to  $\pm 0.0005$  g) of one leaf species was added to covered 1-litre plastic containers with 500 ml of deionised water and 10 ml of tree hole water as a microbial inoculum. Three days after adding the leaves, each container received zero, five, or 15 scirtids (*Helodes* sp.; individual wet masses ranged from 0.0020 to 0.0060 g) collected from tree holes at Merwin Nature Preserve (25 km north-east of Normal, Illinois; 40°30'51"N, 88°59'26"W). These densities approximate the range observed in the field (Daugherty & Juliano, 2001). The following day, zero or 70 synchronously hatched first-instar *O. triseriatus* larvae (F1 progeny of field-collected individuals) were added to containers. Each combination of mosquito density, scirtid density, and leaf type was replicated three times ( $n=72$  microcosms). Containers were housed in an environmental chamber at 25 °C with a LD 16:8 h photoperiod. One and 2 weeks after the initial addition of leaves, an additional 0.1666 g of dried leaf fragments of the same leaf species was added to each container, for a total of  $\approx 0.66$  g per container. Containers were checked every 2 days for dead scirtids (which were removed and replaced) and mosquito pupae, until all mosquitoes either pupated or died. For each eclosed mosquito, sex and dry mass (>2 days at 60 °C) were determined to the nearest 0.0001 mg.

Five correlates of *O. triseriatus* population growth were determined: survivorship (emerged adults/70) and mean dry mass and median days to eclosion for each sex. Overall *O. triseriatus* population performance  $\lambda'$  was also evaluated (Léonard & Juliano, 1995; Juliano, 1998), which synthesises information about survivorship, fecundity (mass), and development time into one value that approximates the realised per capita finite rate of increase. Differences in each correlate among scirtid densities and leaf types were tested for using ANOVA and pairwise comparisons. Because of non-normality, the effects on  $\lambda'$  were analysed with randomisation ANOVA and pairwise comparisons (Manly, 1991). The effects of leaf type, mosquito presence, and scirtid density on scirtid mortality (proportion of initial number of scirtids dying within 6 weeks) were also tested for, using randomisation ANOVA and pairwise comparisons.

After 6 weeks, resource processing was determined for all containers without mosquitoes by separating container contents into coarse (>1 mm), fine (1.0–0.15 mm), and very fine (<0.15 mm) particle sizes. Scirtids were removed, and the contents were stirred then filtered through nested 1 mm and 0.15 mm mesh screens. Very fine particles were quantified based on the dry residue of a subsample of approximately one-quarter of the total volume of filtered liquid ( $\approx 150 \pm 50$  ml). The subsample volume ( $\pm 1$  ml) and the remaining volume were determined, then the subsample

was dried. The residues on 1 mm (coarse) and 0.15 (fine) screens were also collected and dried. Dry masses ( $> 2$  days at  $60^{\circ}\text{C}$ ) of all three size classes were measured to the nearest 0.0001 g. Multivariate analysis of variance and multivariate contrasts were used to test for differences in the proportion of original leaf mass recovered as coarse particulate organic matter and fine particulate organic matter among leaf types and scirtid densities. Fine and very fine components were pooled because fine particles were a minor component in all containers ( $\ll 10\%$  the mass of the very fine component) and showed the same trends as the very fine component.

A correlation between consumer-independent and consumer-dependent processing was tested for because of the potential for such a correlation to modify the predicted effects of manipulating consumer-independent processing rate. Consumer-independent processing was quantified as the mean proportion of original leaf mass consisting of fine + very fine particles for each leaf type in zero-scirtid replicates; consumer-dependent processing was quantified as the difference in mean amount of fine + very fine particles produced in 15- and five-scirtid replicates for each leaf type ( $\text{mean}_{\text{F+VF}(15 \text{ scirtid})} - \text{mean}_{\text{F+VF}(5 \text{ scirtid})}$ ). The loss of coarse particles was used as an alternative measure of resource processing. A correlation was tested for between the proportional amount of coarse particles lost in zero scirtid replicates ( $1 - \text{mean}_{\text{Coarse}(0 \text{ scirtid})}$ ) and the difference in proportional amount lost between 15- and five-scirtid replicates [ $(1 - \text{mean}_{\text{Coarse}(15 \text{ scirtid})}) - (1 - \text{mean}_{\text{Coarse}(5 \text{ scirtid})})$ ]. Pearson correlation coefficients were estimated and tested for significance using a *t*-test (SAS Institute Inc., 1990).

### Physical processing

Leaves of one species were crushed into fine particles in order to manipulate consumer-independent processing rate. This manipulation, though not obviously analogous to any natural phenomenon in container communities (but see Heard *et al.*, 1999), eliminates the correlation of consumer-dependent and consumer-independent processing rates that occurs when manipulating leaf species (see Results). The presence of scirtids and mosquitoes was also varied, with five replicates of each combination of scirtid presence/absence, and mosquito density, and three levels of physical processing ( $n = 60$  microcosms).

Senescent sugar maple leaves were collected from the forest floor during autumn 1998, washed and dried, then stored dry until the following summer. Leaves were soaked in water for 24 h to remove abundant tannins, leaf blades were broken into 10–15 cm<sup>2</sup> fragments, and the fragments were dried for 2 days at  $60^{\circ}\text{C}$ . Allotments of 0.5000 g of leaf fragments (measured to within 0.0050 g) were added to 1-litre plastic containers filled with 500 ml of deionised water and 10 ml of tree hole water. Containers were housed in an environmental chamber at  $26^{\circ}\text{C}$  with a LD 16:8 h photoperiod. Two days after the addition of leaves, either zero scirtids or a mixture of 20 *Helodes*

(mass range =  $0.0040 \pm 0.0020$  g) and five *Prionocyphon* (mass range =  $0.0075 \pm 0.0025$  g) was added to each combination. These densities and species compositions approximate what has been observed in the field. The next day, zero or 100 synchronously hatched first-instar *O. triseriatus* were added to each container.

Beginning 2 days after the initial addition of leaves, an electric blender was used for 3 consecutive weeks to reduce 0, 20, or 50% of coarse particulate organic matter to fine particles. Coarse particles in the 20 and 50% replicates were dried using a paper towel, weighed to the nearest 0.0010 g, and an appropriate portion was processed in the blender with a portion of the container water (free of mosquitoes and scirtids) for 1 min. Leaves in the 0% treatment were dried using a paper towel but were not blended.

Leaves were added to containers 2 (0.25 g), 4 (0.25 g), 8 (0.5 g), and 16 (0.2 g) days after the first addition, for a total of 1.70 g of leaf litter in each container. Containers were checked every 2 days, dead scirtids were removed and replaced, and scirtid exuviae were counted and removed. Eclosing adult mosquitoes were dried and weighed to the nearest 0.0001 mg. At 60 days, mosquitoes were counted by instar, dried for 1 day, and total biomass of larvae in a container was determined to the nearest 0.0001 mg.

The effects of scirtids and blending on mosquito survivorship (proportion alive or pupated at 60 days) and biomass (sum of dry masses of all individuals alive or pupated by 60 days) were analysed using MANOVA (SAS Institute Inc., 1990) and multivariate pairwise contrasts. Survivorship and total mass were squared to meet the assumption of homogeneous variances. The effects of blending and mosquitoes on scirtid mortality (proportion of initial number dying within 30 days) and development (number of exuviae removed by 30 days) were analysed by MANOVA and multivariate pairwise contrasts.

Beginning 1 month after the first addition of leaves, methods similar to those for the leaf manipulation experiment were used to determine resource processing rates. Leaf detritus was sorted into coarse ( $\geq 1$  mm) and fine ( $< 1$  mm) components. The fine component was not subdivided further into a very fine component because of the results of the previous experiment. One replicate was filtered every 5 days, so that all five replicates of a treatment combination were assayed for coarse and fine particles over a 20-day period.

Differences in resource processing rates (proportion of coarse particulate organic matter and fine particulate organic matter) among blending and scirtid treatments were tested for using separate ANCOVAs and pairwise comparisons of least squares means (SAS Institute Inc., 1990), with day of filtering as the covariate. Scirtid conversion of coarse particles into fine particles should be manifest either as a significant scirtid effect or a significant scirtid-day interaction. Because blending treatments were completed before the containers were filtered, blending should not interact with the covariate, so these interactions were omitted.

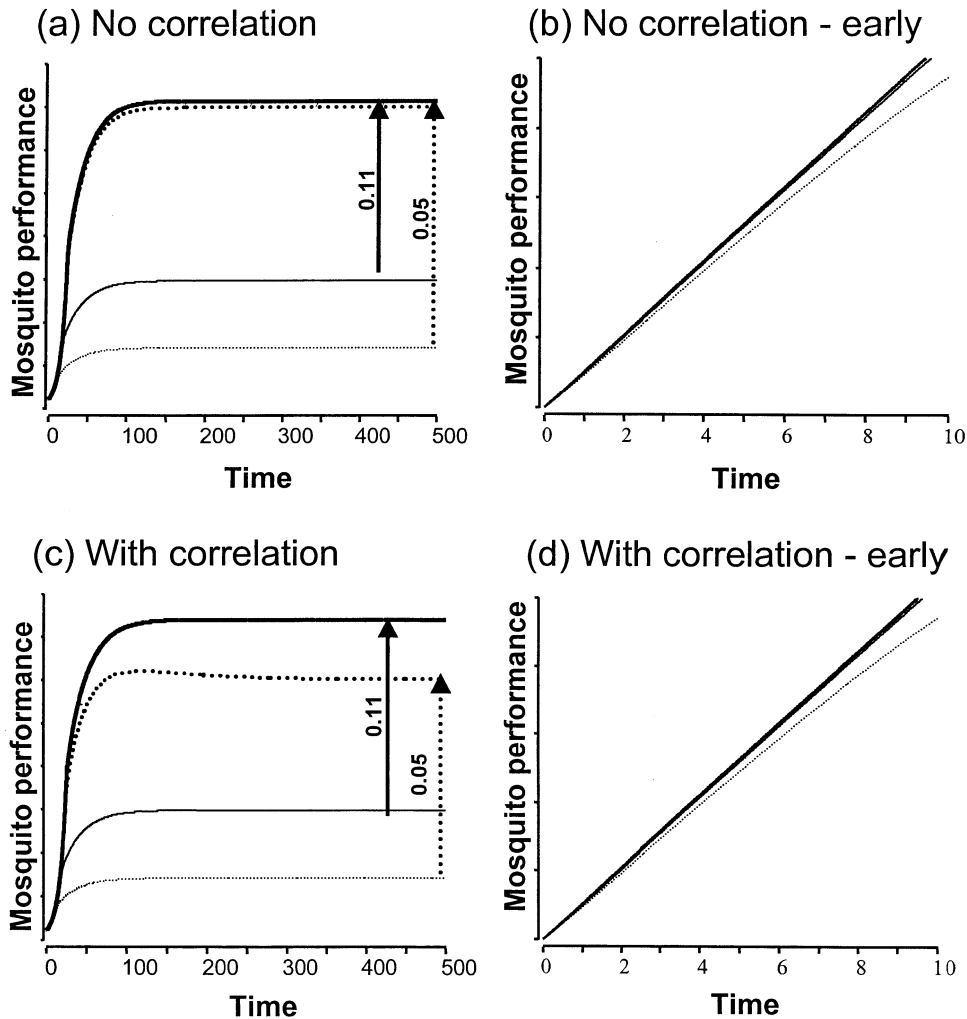
## Results

### Numerical simulations: transient dynamics and consequences of correlation

Context-dependence that was evident at equilibrium (Fig. 2a; note differences in sizes of vertical bars) was also evident in the short term (Fig. 2b; note the large difference in slopes at low consumer-independent processing rate, compared with the very similar slopes at high consumer-independent processing rate). Thus, the upstream consumer

benefited the downstream consumer less at higher consumer-independent processing rates from the outset.

There was an effect of correlation between upstream consumer feeding rate and consumer-independent processing rate on context-dependence but it depended on time frame. If upstream consumer feeding increased with consumer-independent processing rate, upstream consumers benefited downstream consumers to approximately the same degree at equilibrium for all consumer-independent processing rates (Fig. 2c; note similar sizes of vertical bars); however the correlation did not eliminate context-dependence in the



**Fig. 2.** Downstream consumer population growth curves from numerical simulations of a processing chain model with (top two thick lines) and without (bottom two thin lines) an upstream consumer, at different consumer-independent processing rates [ $h=0.05$  (.....) and  $0.11$  (—)]. (a) and (c) depict equilibrium population densities, while (b) and (d) show the short-term dynamics (on a log scale for clarity). (c) and (d) incorporate a positive correlation between upstream consumer feeding rate and consumer-independent processing rate. Vertical bars in (a) and (c) show the magnitude of the beneficial effect of upstream consumers on downstream consumers at each consumer-independent processing rate. Note that the bars are of different lengths in (a) (i.e. context-dependence) and are of similar lengths (i.e. no context-dependence) in (c). In (b) and (d), the difference in slopes for the dotted lines ( $h=0.05$ ; the line with upstream consumers is obscured behind the thick, solid line) is much greater than the difference in slopes for the solid lines ( $h=0.11$ ), suggesting context-dependence in the short-term dynamics, even with a correlation between consumer-independent and consumer-dependent processing.

short term (Fig. 2d; note the same context-dependence seen in Fig. 2b).

#### Multiple leaf types

Leaf species affected the mosquito composite index of performance ( $\lambda'$ ; d.f. = 3,24, %SS = 58.22,  $P < 0.001$ ) and survivorship ( $F_{3,24} = 1029.37$ ,  $P < 0.001$ ) significantly, but the effects of scirtids and the scirtid–leaf type interaction on the composite index of performance (d.f. = 2,24, %SS = 2.24,  $P = \text{NS}$ ; d.f. = 6,24, %SS = 12.51,  $P = \text{NS}$  respectively) and survivorship ( $F_{2,24} = 0.98$ ,  $P = \text{NS}$ ;  $F_{6,24} = 1.47$ ,  $P = \text{NS}$  respectively) were not significant. Masses and development times of both sexes were affected significantly by leaf type (Table 1). Elm was more favourable for mosquito growth than maple, and maple was more favourable than oak or sycamore (Table 2). The only measure of mosquito performance that was affected significantly by scirtids was female development time (Table 1), and development times of both sexes were affected significantly by a leaf–scirtid interaction (Table 1). Development times were shortest with elm, slightly longer with maple, and longest with sycamore and oak. Although mosquito development times were similar for all scirtid densities with elm, maple, and sycamore, development times with oak differed among the scirtid densities (Fig. 3), and the nature of this statistical interaction was different for males and females. Males had longer development times with oak in the 15-scirtid treatment compared with when scirtids were absent (Fig. 3a), whereas females had shorter development times with oak when scirtids were present (Fig. 3b). Scirtid mortality was affected significantly only by leaf species (%SS = 32.16, d.f. = 3,32,  $P < 0.001$ ) and was greater with oak than with the other three leaf species, which did not differ (Table 2).

Both leaf species (Pillai's trace = 9.04, d.f. = 6,52,  $P < 0.001$ ) and scirtid density (Pillai's trace = 4.46, d.f. = 4,52,  $P < 0.01$ ) affected resource processing significantly but there was no interaction between the two (Pillai's trace = 0.91, d.f. = 12,52,  $P = \text{NS}$ ). Elm was processed more quickly than maple, which was processed more quickly than oak and sycamore (Fig. 4a). The proportion of fine particulate organic matter was greater (and coarse

particulate organic matter less) for 15 scirtids than for zero or five scirtids, which did not differ significantly (Fig. 4b).

Correlations between consumer-independent and consumer-dependent processing across leaf types were positive, with high processing in elm and low processing in oak and sycamore. This correlation was not significant when measured as amount of fine particles produced ( $r = 0.907$ ,  $P = \text{NS}$ ) but was highly significant when measured as amount of coarse particles lost during the experiment ( $r = 0.990$ ,  $P < 0.01$ ).

#### Physical processing

Although <5% of the mosquitoes reached adulthood, mosquito survivorship and total biomass at 60 days were affected significantly by blending (Pillai's trace = 10.17, d.f. = 4,46,  $P < 0.001$ ) and scirtid treatment (Pillai's trace = 8.29, d.f. = 2,22,  $P < 0.01$ ), but not by the blending–scirtid interaction (Pillai's trace = 1.61, d.f. = 4,46,  $P = \text{NS}$ ). Mosquito survivorship and mass were greater in containers with 50% blending of leaves than in containers with 20% blending, both of which were greater than the 0% treatment (Fig. 5). Mosquito performance was significantly greater when scirtids were present (Fig. 5).

Scirtids were affected significantly by blending (Pillai's trace = 3.07, d.f. = 4,48,  $P < 0.05$ ) and mosquitoes (Pillai's trace = 5.10, d.f. = 2,23,  $P < 0.05$ ), but not by the blending–mosquito interaction (Pillai's trace = 2.01, d.f. = 4,48,  $P = \text{NS}$ ). There were no significant pairwise differences among blending levels (Fig. 6a). Mosquitoes inhibited scirtids, primarily through a decreased number of moults (Fig. 6b).

Coarse leaf material was affected significantly by blending ( $F_{2,23} = 373.99$ ,  $P < 0.001$ ), scirtids ( $F_{1,23} = 19.57$ ,  $P < 0.001$ ), and filtering day ( $F_{1,23} = 36.34$ ,  $P < 0.001$ ) but not by a blending–scirtid interaction ( $F_{2,23} = 1.23$ ,  $P = \text{NS}$ ). The amount of fine particles was also affected significantly by blending ( $F_{2,24} = 48.25$ ,  $P < 0.001$ ) but not by time ( $F_{1,24} = 3.13$ ,  $P = \text{NS}$ ), scirtids ( $F_{2,24} = 0.53$ ,  $P = \text{NS}$ ), or their interaction ( $F_{2,24} = 0.59$ ,  $P = \text{NS}$ ). The amount of coarse material declined through time (slope  $\pm$  SE =  $-0.0040 \pm 0.0007$ ). The 0% blending treatment had more

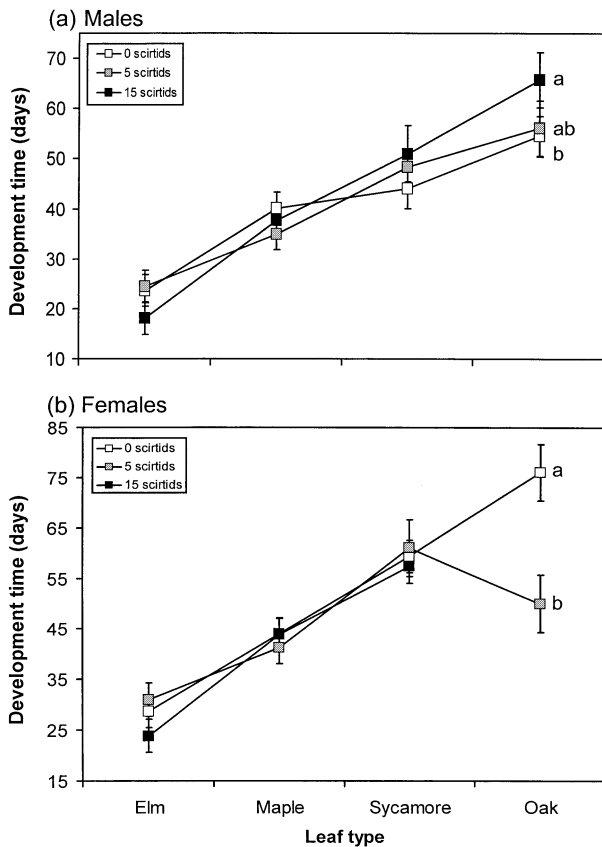
**Table 1.** Effects of leaf species and scirtid density on *Ochlerotatus triseriatus* performance correlates. Each correlate was analysed in separate two-way ANOVAs. Development times were squared in order to meet test assumptions. Common transformations of male mass did not meet these assumptions and caution should be used when interpreting the effects on this correlate.

Source	Males					Females				
	d.f., d.f.e.	Mass		Development time		d.f., d.f.e.	Mass		Development time	
		F	P	F	P		F	P	F	P
Leaf	3,16	23.67	0.0001	70.81	0.0001	3,16	51.91	0.0001	71.06	0.0001
Scirtid	2,16	0.50	0.6141	2.49	0.1122	2,16	0.15	0.8597	7.79	0.0043
Leaf $\times$ scirtid	6,16	0.79	0.5904	2.94	0.0371	5,16	0.96	0.4731	6.33	0.0020

**Table 2.** *Ochlerotatus triseriatus* and scirtid performance (means  $\pm$  SE for  $\lambda'$  and scirtid mortality, least squares means  $\pm$  SE for all others) among leaf types. Means for a variable followed by different letters are significantly different [sequential Bonferroni correction, experiment-wise significance ( $\alpha_e$ ) = 0.05]. Female mosquito mass was not estimable (NA) for oak treatments due to a lack of female eclosion.

Leaf type	<i>Ochlerotatus triseriatus</i>				Scirtid
	Composite index of performance, $\lambda'$	Percentage survivorship	Male mass (mg)	Female mass (mg)	Percentage mortality
Elm	1.083 $\pm$ 0.006 a	0.856 $\pm$ 0.019 a	0.251 $\pm$ 0.010 a	0.542 $\pm$ 0.016 a	0.078 $\pm$ 0.026 a
Maple	1.050 $\pm$ 0.002 b	0.738 $\pm$ 0.018 b	0.242 $\pm$ 0.019 a	0.436 $\pm$ 0.016 b	0.044 $\pm$ 0.019 a
Oak	0.767 $\pm$ 0.145 c	0.024 $\pm$ 0.018 c	0.136 $\pm$ 0.019 b	NA	0.244 $\pm$ 0.060 b
Sycamore	0.222 $\pm$ 0.147 c	0.035 $\pm$ 0.018 c	0.154 $\pm$ 0.019 b	0.241 $\pm$ 0.020 c	0.044 $\pm$ 0.019 a

coarse particles and fewer fine particles than did the 20% blending treatment, which had more coarse particles and fewer fine particles than the 50% blending treatment (Fig. 7a). Containers with scirtids had less coarse particulate organic matter remaining than did those without scirtids (Fig. 7b).



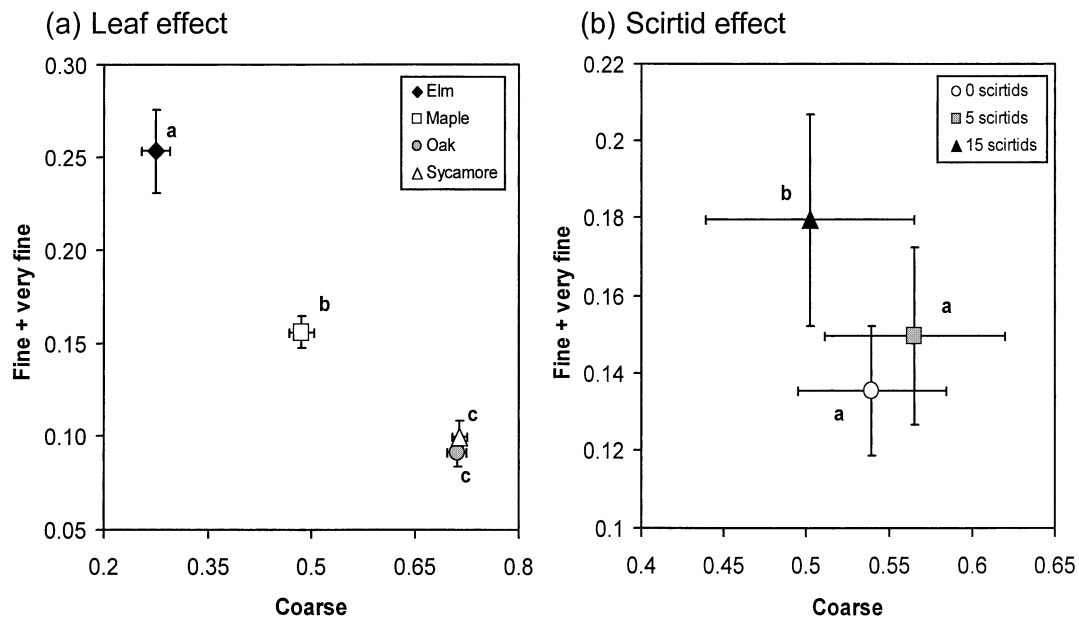
**Fig. 3.** Least squares mean development times for (a) males and (b) females as a function of leaf type and scirtid density. Means for different scirtid densities within the oak treatment followed by different letters differ significantly (Bonferroni,  $\alpha_e$  = 0.05). Pairwise comparisons among scirtid densities within the other three leaf types were not significant. Error bars denote  $\pm$ 1 SE.

## Discussion

One of the main predictions of processing chain models is that the effect of the upstream consumer on the downstream consumer shifts from beneficial to harmful as the rate of consumer-independent processing increases. In both experiments, the manipulations of consumer-independent processing were successful, producing significant effects on the production of fine particles (Figs 4 and 7) and on mosquito performance (Table 2, Figs 3 and 5). Furthermore, as in previous studies (Paradise & Dunson, 1997; Paradise, 1999, 2000), scirtids increased the breakdown of coarse leaf litter and, more importantly, it appears that scirtids may increase the production of fine particles. Thus, the ecological context in which scirtids and mosquitoes interact was manipulated successfully and scirtids appear to have the potential to affect mosquitoes via resource processing. In both experiments, however, the predicted statistical interaction of scirtid density and consumer-independent processing (either leaf type or physical processing) on mosquito performance was largely non-significant.

The prediction that increased consumer-independent processing rate should lead to more negative effects of scirtids on mosquitoes comes from eqn 1. Because the conditions for eqn 1 were derived for equilibrium (Heard, 1994a), it may be inappropriate to draw conclusions about context dependence in a non-equilibrium experimental system like these mosquitoes and scirtids. Heard (1995) may have offered indirect support for context-dependence in short-term dynamics by showing that higher consumer-independent processing rates cause switches from commensalism to amensalism to occur earlier than at low consumer-independent processing rates, at time horizons well before equilibrium. More direct evidence for context-dependence in short-term dynamics was obtained from the numerical simulations in this study, which showed context-dependence at very early time horizons (Fig. 2). This result is important for two reasons. (1) It fills in a gap in processing chain theory. (2) Because the conditions thought to influence context-dependence (i.e. consumer-independent processing rate, sloppiness, and upstream loss rate) at equilibrium are also important in the short term, this result suggests that it is appropriate to use a non-equilibrium



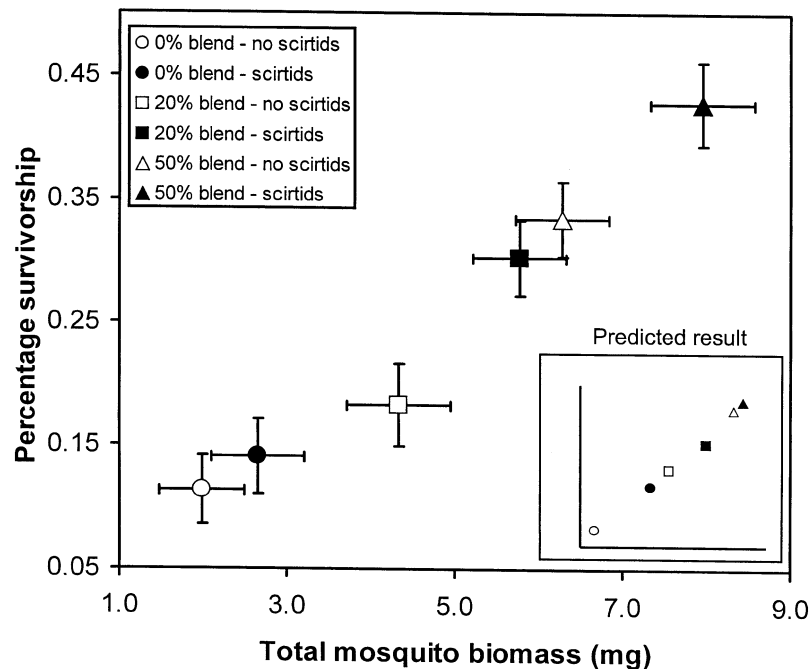


**Fig. 4.** Effects of (a) leaf species and (b) scirtids on resource processing. The amount of coarse material and the fine + very fine particles remaining are shown as proportions of original leaf mass. Different letters denote significant differences among treatment combinations.

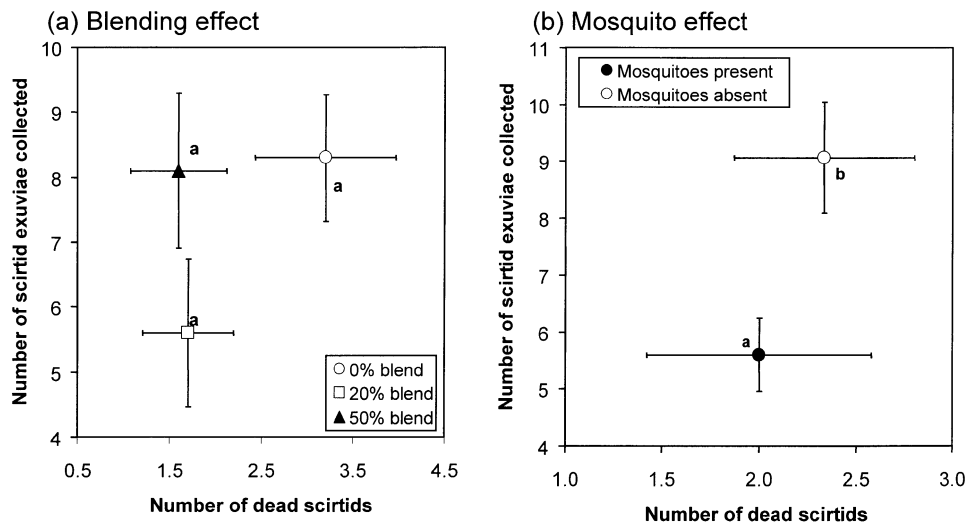
experimental system to test the hypothesis that context-dependence is governed by resource processing rates.

The other important result from the numerical simulations is that a positive correlation between upstream

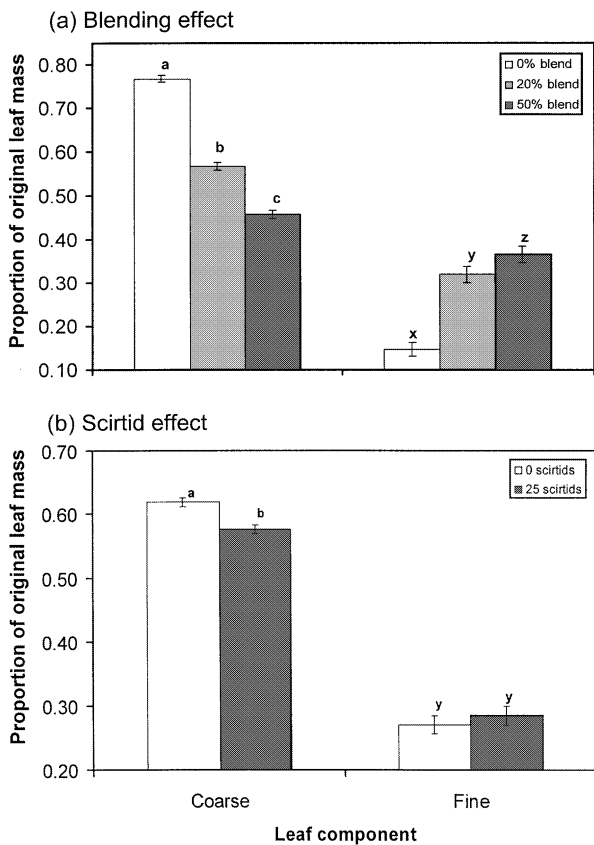
consumer feeding and consumer-independent processing rate has the potential to weaken context-dependent shifts in upstream consumer effects on equilibrational downstream consumer dynamics (Fig. 2c). This suggests that such a



**Fig. 5.** Effects of blending and scirtids on percentage survivorship and total biomass of mosquitoes. There were significant effects of blending and scirtids but no blending–scirtid interaction. Bivariate means ( $\pm 1$  SE) for each blending–scirtid combination show that the type of blending–scirtid statistical interaction predicted by the model (depicted in the box in the lower right-hand corner) did not occur. All bivariate pairwise comparisons between blending levels (across scirtid treatments) were significant ( $50\% > 20\% > 0\%$ ; Bonferroni correction,  $\alpha_c = 0.05$ ).



**Fig. 6.** Effects of (a) blending and (b) mosquitoes on the numbers of dead scirtids and scirtid exuviae. Bivariate means ( $\pm 1$  SE) followed by different letters are significantly different (Bonferroni correction,  $\alpha_c = 0.05$ ).



**Fig. 7.** Effects of (a) blending and (b) scirtids on the amounts of coarse material and fine particles at the end of the experiment, expressed as proportion of original leaf mass. Least squares means ( $\pm 1$  SE) for coarse or fine material followed by different letters are significantly different (Bonferroni correction,  $\alpha_c = 0.05$ ).

correlation makes it difficult to test context-dependence by manipulating the species of leaf. Further, it implies that context-dependent shifts from commensal to amensal outcomes are unlikely to occur in nature in response to different leaf species, which are probably the main source of natural variation in consumer-independent processing rate in tree holes. In the leaf manipulation experiment, the only evidence that scirtids benefited mosquitoes differentially was more rapid female development in the oak treatment (Fig. 3b), which is not compelling evidence of context-dependent outcomes mediated by leaf species. Slowly decomposing leaves, like oak and sycamore, provide a poorer resource for mosquitoes than do quickly decomposing leaves, such as maple (Fish & Carpenter, 1982). The potential for fine particles produced by scirtids to benefit mosquitoes should be greatest in containers with slowly decomposing leaves; however if slowly decomposing leaf types are also less consumable for scirtids, this predicted statistical interaction (leaf species  $\times$  scirtid) is less likely. There was a strong, positive correlation between leaf decay rate and scirtid consumption. Oak and sycamore leaves are thicker, tougher, and waxier than elm and maple leaves (M. P. Daugherty, pers. obs.), suggesting that the physical structure of leaves may affect the ability of scirtids to convert them into fine particulate organic matter available to mosquitoes. Thus, manipulating leaf type not only alters consumer-independent processing rate but also consumer-dependent processing (of which sloppiness is a component) and possibly resource loss rate (i.e. all three of the parameters in eqn 1). If these three parameters are correlated positively, manipulating leaf type may not reverse the inequality in eqn 1 and will not lead to a shift from commensal to amensal.

It is important to note, however, that the effect of a correlation between upstream consumer feeding and consumer-independent processing rate on the short-term

dynamics may be minimal (Fig. 2d). Given that dynamics of mosquito populations may often be non-equilibrium in the field (Bradshaw & Holzapfel, 1983, 1985, 1988), the lack of evidence for context-dependence in the leaf manipulation experiment may be explained better by a second possibility involving the great differences in mosquito performance among the leaf types. There was a 35-fold difference in the average mosquito survivorship to adulthood among the leaf types. Several replicates in the oak treatment did not produce any adult females (Table 2). Such great differences among leaf types coupled with limited replication associated with the factorial design of the experiment may have made detecting potentially subtle effects of scirtids difficult. In the leaf manipulation experiment, scirtids did not affect mosquitoes even though they increased the loss of coarse particles and production of fine particles. Even if the correlation between leaf decay rate and consumer-dependent processing could explain the lack of context-dependence in response to different leaf types, it would not explain why scirtids had relatively weak effects on mosquito performance (Fig. 2c; note that commensalism is evident despite the correlation). In effect, great differences among leaf types may have swamped the effects of scirtids and therefore limited the possibility of detecting context-dependent effects of scirtids on mosquito performance. The limited benefit of scirtids to mosquitoes may also arise because scirtids themselves were affected by manipulations of consumer-independent processing and by mosquitoes. Scirtid mortality was significantly greater with oak than with the other leaf types, and blending affected scirtid mortality and development. In addition, mosquitoes seem to have slowed scirtid development rate. If scirtid development or survival is compromised, it is likely that their consumption of coarse particles, and therefore their effect on other organisms, will decrease.

The blending experiment was designed to eliminate qualitative differences among leaf species, thereby enabling the manipulation of consumer-independent processing rate without altering consumer-dependent processing rate; however, although scirtids did benefit *O. triseriatus* in this experiment, the effect was not dependent on the blending treatment. There are two issues that require resolution before concluding that the lack of the predicted statistical interaction (blending  $\times$  scirtid) in this experiment refutes the model's prediction that processing chain dynamics are governed by consumer-independent processing rate. First, an interaction might have been detected if the experiment had been followed through to adult mosquito eclosion. This seems unlikely. Not only were larval survivorship and mass not affected significantly by the statistical interaction but there was a trend for scirtids to facilitate mosquitoes *more* in the blending (20 and 50%) treatments than in the no blending (0%) treatment (Fig. 5; compare predicted result in the inset with the observed result in the graph; note differences between solid and open symbols of the same shape), which is the opposite of the model's prediction. The second issue is that mosquitoes affected scirtid performance negatively (Fig. 6b). Heard's (1994a, 1995)

model of a two-species processing chain postulates unidirectional biotic effects from upstream consumer to downstream consumer. Slower development of scirtids in the presence of mosquitoes is inconsistent with this model structure, suggesting that interactions between scirtids and *O. triseriatus* are more complex than a simple upstream-downstream relationship. Paradise (2000) noted a similar negative effect of mosquitoes on scirtid mass, which he attributed to behavioural interference of mosquitoes on scirtid feeding (see also Heard, 1994b). Another possibility is chemical interference. Mosquitoes may affect one another via excreted waste products (Moore & Whitacre, 1972; Walker *et al.*, 1991), however the effects of mosquito excretory metabolites on other container insects have not been documented. Perhaps a more likely explanation is that the feeding modes of *O. triseriatus* and scirtids may overlap. Scirtids are thought to be scrapers, using their mandibles to remove microbes attached to surfaces (Barrera, 1996) but the consumption of fine particles by scirtids has not been studied. *Ochlerotatus triseriatus* larvae are primarily filter feeders but they browse the surfaces of leaves for attached micro-organisms regularly (Barrera, 1996), and when food is scarce they spend more time browsing (Juliano *et al.*, 1993). Thus, scirtids and mosquitoes may feed on the same resource (micro-organisms attached to leaf surfaces or leaf fragments) but with varying degrees of spatial and temporal partitioning. This overlap in resource use may result in competition when resources are limiting (e.g. when consumer-independent processing is low). If so, the potential for scirtids to facilitate mosquitoes is not as great as believed previously, and perhaps other members of container communities should be investigated in order to test for context-dependent processing chain interactions with scirtids (i.e. strict filter-feeders such as *Culex* mosquitoes). Whatever the mechanism of the negative effects of mosquitoes on scirtids, these effects are inconsistent with existing models (Heard, 1994a, 1995), so those models are unlikely to describe the scirtid-mosquito system adequately.

If processing chains are as common as suggested (Heard, 1994a), these interactions may help in understanding the forces that structure a broad range of ecological communities. Processing chains may provide unique insight into the types of factors that contribute to context-dependence and the likely implications of variability in these factors. These experiments are the first rigorous test of whether species interact in the context-dependent manner predicted by processing chain theory, because one of the parameters that is predicted to determine the nature of processing chain interactions was manipulated directly. There was not, however, strong evidence of context-dependent biotic interactions that are governed by resource processing rates. Paradise (1999, 2000) found that the effects of scirtids on *O. triseriatus* depend on ecological context (leaf amount or pH) but his tests did not address mechanisms of context-dependence within processing chain models explicitly, and therefore offer no better support for the predictions of processing chain models. The results

presented here suggest that current models of processing chains do not describe scirtid–*O. triseriatus* interactions accurately, probably because biological and ecological characteristics of scirtids, *O. triseriatus*, and leaves are not incorporated explicitly into these existing models. These differences are probably sufficient to require more sophisticated models of leaf processing in this system. Other pathways of interaction between mosquitoes and scirtids must be built into models of this system if they are to predict interactions between these species. Finally, the generality of these results with respect to processing chain interactions as a whole needs to be determined via similar experiments on other processing chain systems in order to determine whether current models describe their dynamics adequately.

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