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Resource limitation, habitat segregation, and species interactions of british tree-hole mosquitoes in nature

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Summary. The insect fauna of water-filled tree holes in southern Britain consists primarily of the mosquitoes *Aedes geniculatus, Anopheles plumbeus, Culex torrentium,* and a benthic detritivorous fauna that includes primarily the scirtid beetle *Prionocyphon serricornis* and the chironomid midge *Metriocnemus martinii. Culex torrentium* has been documented only relatively recently in tree holes but all three species of mosquitoes partition the resource in space and time. When mosquito larvae were forced to coexist in natural tree holes at limiting densities and at higher than natural levels of interspecific encounter, there was no evidence that *Aedes geniculatus* or *Anopheles plumbeus* affected pupation success, pupal weight, or development time of the other or that either *Aedes geniculatus* or *C. torrentium* affected the survivorship, pupation success, pupal weight, and biomass yield of the other. When *A. geniculatus* at limiting densities were forced in natural tree holes to live without or to coexist with natural or twice natural densities of *P. serricornis* and *M. martinii,* the presence, absence, or superabundance of the benthic insects did not affect pupation success or pupal weight of *A. geniculatus;* development time of *A. geniculatus* was faster when a superabundance of the benthic fauna was present. Effects of the benthic fauna on *A. geniculatus* are slight and the only significant interaction is facilitative, not competitive. The pattern of habitat segregation among tree-hole mosquitoes in southern Britain is characteristic of their respective genera and we propose that this pattern is more likely (but not certain) to have arisen through a process of independent evolution than through competitively driven niche shifts among already coexisting species.

Key words: Competition – Facilitation – Communities

The concept of the struggle for existence, fundamental to Darwinian selection, has long implied that species compete for resources both among themselves and with other species. Conventional wisdom would dictate that the potential for competition should be acute in confined spaces. Hutchinson (1959) did not choose to examine the cohabitation of 20 or more species of corixid bugs that may occur in a 1-acre pond; rather, he composed his "Homage to Santa Rosalia" after reflecting upon the coexistence of only two species in a small limestone spring. Even in more restricted habitats such as plant stems (Rathcke 1976), rolled *Heliconia* leaves (Strong 1982), *Heliconia* flower bracts (Seifert 1982), cattle dung (Holter 1982), mushrooms (Atkinson and Shorrocks 1984), dead *Cepaea* snails (Beaver 1977), or the rot holes of trees (Bradshaw and Holzapfel 1983; Lounibos 1985), competition between species is the exception rather than the norm and facilitative interactions are as frequent as competitive interactions. In the last case, competition among tree-hole mosquitoes in southeastern North America is prevented to a large extent by a dominant, self-limiting larval predator. The goal of this study is to examine the interactions among tree-hole mosquitoes that have evolved in the absence of a major larval predator.

The usual approach to analyzing the role of predation on community structure is to remove the predator from some subset of habitats and then to compare the response of prey populations in the presence and absence of predation (Connell 1983; Schoener 1983; Sih et al. 1985). This approach assumes that all variation in the responses of the prey to the predator is environmental and not genetic. Otherwise, even in the absence of the predator, prey populations may continue to behave as if the predator were present. For this reason, the current paper investigates interspecific interaction among three species of coexisting tree-hole mosquitoes in western Europe where larval predators have been absent through at least recent evolutionary time (Kitching 1971; Marshall 1938; Rohnert 1950; Rioux 1958).

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Tree holes are especially appropriate habitats for comparative studies of population interactions. Their range extends from the subarctic to the equator; they are ubiquitous in and harbor insects at all latitudes where hardwood trees occur, even in the Mojave Desert (Zavortink 1985). Over this range, these habitats exhibit tremendous variation in the surrounding climatic conditions, species composition, and community complexity. Fisher et al. (1990) found that interspecific interactions of tree-hole *Aedes* were not affected by differences in tree-hole detritus between North American rot holes and British buttress (pan) holes. Tree holes therefore provide excellent replicate habitats for the study of population interactions over geographic, even intercontinental, distances.

Throughout the world, the most frequent, consistent inhabitants of tree holes are mosquitoes, many of which are specific to that habitat. In southeastern North America, five species of mosquitoes coexist in tree holes. These five species partition their confined habitat by trophic specialization, by utilization of transient or persistent tree holes, and by vertical stratification within tree holes (Bradshaw and Holzapfel 1983). The role of habitat segregation in permitting coexistence is, however, obscured by the observation that species in the community are limited more by drought and predation than by resource availability (Bradshaw and Holzapfel 1983, 1984, 1985, 1988; Lounibos 1983, 1985). Even in more northern tree holes in the absence of larval predators, the non-predatory species maintain similar oviposition patterns. In the north, these patterns may be maintained by drought and winter cold as much, if not more, than by resource limitation (Copeland and Craig 1990a, b).

In southern Britain, three species of mosquitoes are most prevalent in tree holes: *Aedes geniculatus* (Olivier), *Anopheles plumbeus* Stephens, and *Culex torrentium* Martini (Marshall 1938; Yates 1979; Bradshaw and Holzapfel 1986). These three species exhibit distinct habitat segregation (Bradshaw and Holzapfel 1986). *Anopheles plumbeus* occupies permanent rot holes in a variety of tree species and may also extend habitat usage into deeper, more cryptic buttress holes (= pans, sensu Kitching 1971) in beech trees. *Aedes geniculatus* occupies rot holes and cryptic buttress holes along with *Anopheles plumbeus* but also abounds in the largest, most exposed buttress holes. *Culex torrentium* occupies primarily the exposed buttress holes. In addition to these modes of macro-habitat segregation, *A. plumbeus* exhibits micro-habitat specialization by being an efficient surface filter feeder while the remaining species are filter feeders and browsers in the water column of tree holes. *Orthopodomyia pulchripalpis* (Rondani) has been recorded from Britain (Beattie and Howland 1929; Fallis and Snow 1983; Macan and Tutin 1932) but this species is now rare and we never encountered it, even at Burnham Beeches in 1984 or 1986.

Finally, the mosquito fauna is typically accompanied by a benthic, detritivorous fauna (Kitching 1971) that commonly includes *Metriocnemus martinii* Thien. (Diptera: Chironomidae), *Prionocyphon serricornis* Müller (Coleoptera: Scirtidae), *Myiatropa florea* L. (Diptera:

Syrphidae), and *Dasyhelea dufouri* Laboulbène (Diptera: Ceratopogonidae). Although these species frequently co-occur in tree holes with mosquitoes, interaction between the culicid and the other faunas has never been considered experimentally.

The present study seeks to answer several basic questions about interactions among coexisting tree-hole insects:

1. Is microhabitat segregation between the surface feeding *Anopheles plumbeus* and the water-column feeding *Aedes geniculatus* sufficient to explain their coexistence?
2. Are tree holes an invadable niche for *C. torrentium*; specifically, is there a dominance hierarchy between *C. torrentium* and *A. geniculatus* in the same hole?
3. Does the benthic fauna interact with the mosquitoes and, if so, is the interaction facilitative or competitive?
4. Do the constituent species each encounter limiting resources and, if so, do interspecific interactions exceed intraspecific interactions?
5. Has the pattern of habitat segregation observed now arisen through a process of competitively driven niche shifts among coexisting species or through an independent process by individual species?

Materials and methods

Experimental rationale

The basic approach was to examine intra- and interspecific interactions among as many of the arthropod inhabitants of tree holes as possible within the constraints of time, tree-hole availability, and arthropod abundance. Priority of consideration was given to *Aedes geniculatus*, *Anopheles plumbeus*, *C. torrentium*, and other arthropods, respectively, due to their relative abundance and ease of handling as larvae and to their attraction to human bait as adults.

Interactions were assessed by manipulating larval densities in tree holes to compare performance in different tree-hole types or to force interspecific encounters in space and time. Several components of mosquito development are correlated with overall fitness and provide measures of overcrowding or of resource limitations. Low per capita resources result in retarded larval growth and development, reduced larval survivorship, reduced pupal weight, reduced biomass yield, and lower per capita rates of increase (Hawley 1985a, b; Hard et al. 1989; Fisher et al. 1990; references therein). These components of development were formally defined for each particular species as follows:

1. *Pupation success:* cumulative number of pupae recovered from each hole throughout the experimental period divided by the number of larvae in the original cohort
2. *Pupal weight:* mean pupal weight of each sex of all pupae recovered from each hole during the experimental period
3. *Development time:* mean weeks to pupation of each sex among all pupae recovered from each hole during the experimental period
4. *Survivorship:* (cumulative number of pupae recovered throughout the experiment plus the number of third and fourth instars remaining at the end of the experiment) divided by (number of larvae in the original cohort for *Aedes geniculatus* or number of third and fourth instars in the original cohort for *Anopheles plumbeus*)
5. *Yield:* (cumulative sum of pupal and larval weights at the end of the experiment) divided by the weight of larvae added at the start of the experiment.

Upon the appearance of the first pupa, each hole was sampled once a week for the duration of the experiment. Pupae were re-

moved in the field, transported back to the laboratory, identified, sexed, and weighed to the nearest 0.1 mg on a microbalance. To avoid repeated sampling of the same individuals, pupae were not returned to the field. Pupal weights were obtained directly; larval weights were calculated from the instar distribution at the end of the experiment by the method of Bradshaw (1983) extrapolating from actual wet weights of field-collected fourth instars. To convert wet to dry weight, we used the conversion factor determined for *Aedes sierrensis* in the laboratory:

wet weight (mg) = 0.25 + 4.84 dry weight (mg) $(r^2 = 0.943)$

Sampling

All of the observations and experiments were performed at three main localities, Silwood Park, Blacknest, and High Standing Hill, within 6 km of Ascot, Berkshire, in southeastern England ($51°24'$N, $0°40'$W). A description of these localities and details of sampling techniques are given in Bradshaw and Holzapfel (1991).

All of the experimental densities were based on levels of mean crowding actually observed in 1986. Mean crowding, Z_{xy}, of species Y on species X per litre volume (v_i) in the ith tree hole was calculated as (Lloyd 1967; Hurlbert 1978):

$$Z_{XY} = \Sigma[(x_iy_i)/v_i]/\Sigma x_i \qquad (1)$$

where x_i and y_i are the numbers of species X and species Y, respectively, in the ith hole. Mean intraspecific crowding was calculated by substituting $(x_i - 1)$ for y_i in the above equation. Densities for spring experiments were based on mean crowdings observed in 42 holes prior to manipulation at Silwood Park and at Blacknest. Densities for summer experiments were based on additional mean crowdings observed in 37 unmanipulated holes at High Standing Hill.

Tree holes in the vicinity of Ascot were frozen throughout February and did not thaw until 3 March 1986. Hatching of *Aedes geniculatus* occurred immediately and increased as temperatures rose during March. Forty tree holes at Silwood park were located and censused on 7–11 March and 17 holes at Blacknest on 14 March. Known holes were censused again from 22 to 28 March to monitor the progress of hatching. Although some hatching of *A. geniculatus* was continuing, most of the larvae in tree holes could be attributed to earlier hatching immediately after the thaw. Consequently, mean crowding estimates were made from the 22–28 March census and experimental densities of *Aedes* and *Anopheles* based on these estimates. *Culex torrentium* overwinter primarily as adults but also occasionally as late-instar larvae. The first egg rafts were not observed until July. Densities of *C. torrentium* peaked in late July and experimental densities involving this species were based on mean crowdings estimated from censuses of unmanipulated tree holes at High Standing Hill on 21 to 27 July.

Since the goal was to assess biotic interactions within and among species, water volume in experimental tree holes was maintained at March 1986 levels by the addition of glass-distilled water after each census.

Statistical procedures

All analyses of variance (ANOVA) and regression were performed according to procedures outlined in Sokal and Rohlf (1969). All percentage values in pre-planned experiments were arcsine transformed prior to regression or ANOVA. All ANOVAs were performed on otherwise untransformed data unless Bartlett's test for homogeneity of variance rejected the null hypothesis of homogeneous variances. When Bartlett's test indicated heterogeneous variances, $\log(x)$ or $\log(x + c)$ transformations rendered the variances non-heterogeneous. Two-way ANOVAs that involved unequal and disproportionate sample sizes were performed using the SAS General Linear Model (SAS GLM) procedure (SAS Institute 1985).

Experimental procedures

Interactions between Aedes and Anopheles. The purpose of this experiment was to reveal interspecific interactions between *Aedes geniculatus* and *Anopheles plumbeus* if they existed. The basic design forced each species to encounter more of the other than it would under normal circumstances and then compared the performance of each species when it was reared alone or together with the other species.

The experimental design included 3 treatments with 4 holes per treatment:

    (a) 291 *Aedes geniculatus* alone per litre

    (b) 45 *Anopheles plumbeus* alone per litre

    (c) 291 *Aedes geniculatus* plus 45 *Anopheles plumbeus* together per litre

Experimental density for *Aedes geniculatus* alone was set equal to the average of its own mean crowding and its mean interspecific crowding on *Anopheles plumbeus* (Bradshaw and Holzapfel 1991): $(337 + 246)/2 = 291/l$. Likewise, the density of *Anopheles plumbeus* alone: $(82 + 7)/2 = 45/l$.

The tree holes for this experiment were allocated in such a manner that the tree holes in each treatment did not differ in either the mean or variance of volume. The instar distribution among experimental *Aedes geniculatus* and *Anopheles plumbeus* was determined from the 22–28 March census when *Aedes geniculatus* comprised 0.859 first instars, 0.067 second instars, 0.036 third instars, and 0.038 fourth instars and *Anopheles plumbeus* of 0.156 first instars, 0.307 second instars, 0.450 third instars, and 0.087 fourth instars. After the removal of all residual larvae, the experiment was initiated 31 March and censused from 30 April to 5 May, on 20–21 May, 21 June, 15–16 July, and 4–6 August when the experiment was terminated due to dwindling *Anopheles plumbeus*. Since hatching of *Aedes geniculatus* appeared to be an ongoing process, the density of *A. geniculatus* was adjusted in each hole during the first two censuses to 291/l by the removal or addition of first instars. Added first instars were taken directly from remote, non-experimental holes at Silwood Park and Blacknest. Upon the appearance of *C. torrentium* egg rafts in mid-July, each tree-hole was checked with a torch every 48–72 h and all egg rafts were removed. Pupae of *A. geniculatus* and *Anopheles plumbeus* were removed, identified, sexed, and weighed once per week throughout the experiment.

Performance of *Aedes geniculatus* and *Anopheles plumbeus* in each hole was gauged by pupation success, pupal weight, and development time.

Interactions between Aedes and Culex. This experiment was designed to determine whether there was any competitive dominance between *Aedes geniculatus* and *C. torrentium*. The basic design was to compare the performance of each species when reared alone with its performance when reared with the other species. This experiment was run in beech trees at Silwood Park during the summer and fall when *C. torrentium* hatched into and developed in tree holes (Bradshaw and Holzapfel 1991). Twelve holes of the type normally co-occupied by *Aedes* and *Culex* were chosen and matched; these holes were intermediate between cryptic buttress and exposed buttress holes or were the larger, more exposed of the cryptic buttress holes. The experiment consisted of three treatments with four holes each; holes were assigned to treatements so that the mean and variance of volume did not differ among treatments:

    (a) 400 *A. geniculatus* alone per litre

    (b) 400 *C. torrentium* alone per litre

    (c) 200 *A. geniculatus* plus 200 *C. torrentium* per litre

Experimental densities were set after consideration of mean crowdings in unmanipulated tree holes at High Standing Hill prior to the start of the experiment (Bradshaw and Holzapfel 1991). An experimental density of 400 larvae per litre was selected as an average between the two intraspecific mean crowdings and as a density that would produce higher mean interspecific crowdings than those prevailing in nature.

All larvae were removed from each hole and discarded immediately prior to the start of the experiment. They were replaced with

first instars of *A. geniculatus* and/or *C. torrentium* that had hatched within the previous 24 h. *Aedes* were obtained as the F_1 of adults reared from wild-caught pupae. *Culex* were obtained by accumulating egg rafts from tree holes at Silwood Park and keeping them refrigerated until the day before starting the experiment when they were floated on tubs of water in the laboratory and hatched by the next day.

The experiment was initiated on 7 August 1986. Holes were checked for *Culex* egg rafts 3 times a week and populations were sampled for pupae once a week. Populations were censused on 26 August, 9 September, 23–24 September, 7 October, and, finally, on 21 October. By the last date, all pupation of *Aedes* had ceased and all larvae of *Culex* except one individual had pupated or died. At each census, all larvae of *Anopheles plumbus* were removed.

Aedes geniculatus are able to overwinter as either embryos or fourth instars; *Culex torrentium* overwinter mainly as adults but sometimes as fourth instars. Consequently, performance was estimated by pupation success, by mean pupal weight, by yield, and by survivorship.

Interactions between mosquitoes and other arthropods. To assess competitive or facilitative interactions between mosquitoes and other tree-hole inhabitants, the densities of mosquitoes and the other benthic arthropods were manipulated in tree holes at Blacknest. The objective was to force *Aedes* to encounter extraordinarily low or high densities of the other insects under uniform but resource-limiting conditions. For this experiment, 12 carefully matched cryptic buttress holes were selected over a wide range of volumes and assigned to three treatments that did not differ in the mean or variance of tree-hole volume. The basic design was to remove the total contents of all holes, census all insects, and pool and return the tree-hole contents so that all holes received the same mixture. The insects were re-distributed in three combinations:

(a) *Aedes* alone at a density equal to the mean intraspecific crowding of *Aedes* on itself

(b) *Aedes* at the same density as *Aedes* alone plus all other insects at a density equal to the mean interspecific crowding of each other species on *Aedes*

(c) *Aedes* at the same density as *Aedes* alone plus all other insects at a density equal to double the mean interspecific crowding of each other species on *Aedes*

During 2–6 April 1986, two fractions of the total contents were removed from each hole in the following order: (1) siphonable water and (2) litter consisting of whole leaves, leaf fragments and other large detritus, and fine detritus with residual water.

Tree-hole contents were immediately transported to the laboratory and censused on the day of sampling. After censusing, the litter fraction of each hole was separated in the lab by the following procedure:

Leaf fraction: whole leaves and leaf fragments equal to about 0.25 area of a leaf or larger

Coarse sediment: remaining sediment retained by a 1.8-mm mesh aquarium net

Fine sediment: water and sediment passed by a 1.8-mm mesh aquarium net

The leaf and coarse fractions were each drained, squeezed by hand, and weighed to the nearest gram with a spring scale. After weighing, each fraction was pooled with the same fraction from all other holes. After pooling, each combined fraction was mixed vigorously by hand in a large bucket.

Fractions of tree-hole contents were returned to tree holes on a weight per volume basis so that the holes and their contents were reconstituted to their original total volume. Total leaf plus coarse sediment from all holes divided by total siphonable water from all holes yielded an average of 471 g/l of which the leaf and course fractions comprised 43 and 57%, respectively. To maintain as much of the original stratification as possible, the fractions were added as coarse sediment first, leaf fraction second, fine sediment third, and insects last.

Aedes geniculatus were added at a density of 337 larvae per liter, reflecting their mean intraspecific crowding at Blacknest and Sil-

wood Park (Bradshaw and Holzapfel 1991). The higher combined density of 337/1 was chosen over the local mean crowding of 200/1 to assure a limiting density of *A. geniculatus* and so create maximum sensitivity of *Aedes* populations to variation in the density of other insects. The instar distribution (0.579 first, 0.263 second 0.094 third, and 0.064 fourth) reflected the relative distribution prevailing in control tree holes at the time.

Other insects were added in multiples of their mean interspecific crowding on *Aedes geniculatus* at Blacknest (Fig. 1): 287 *Metriocnemus martinii* per litre and 59 *P. serricornis* per litre. *Myiatropa florea* was omitted because its mean interspecific crowding on *A. geniculatus* was less than unity (0.3/1). Both *Aedes* and other insects were counted out on the day of the experiment, transported to the field in plastic containers with each species segregated in its own container, and added to the holes after the fractions had been reconstituted.

The experiment was set up on 7 April 1986. Holes were sampled weekly for pupae. Holes were censused on 6 May, 22–23 May, 23 June, 17 July, and, finally, on 1 August. Of the 12 holes, only 2 showed an increase in the density of *Aedes geniculatus* and only during the census on 6 May. The density of mosquitoes in the holes that showed an increase was reduced to the starting density but the density of mosquitoes or other insects was not otherwise altered after the start of the experiment either in these 2 or in the remaining 10 holes. At each census, freshly hatched larvae of *Anopheles plumbeus* and *C. torrentium* were removed.

Performance of *Aedes geniculatus* in each hole was estimated from pupation success, pupal weight, and development time.

Results

Mean crowding

Highest mean intraspecific crowdings at Blacknest between 22 and 28 March were observed among *Metriocnemus martinii* and *Aedes geniculatus* (Fig. 1). *Metriocnemus* encountered over 1600 of its own species per litre and *Aedes* 200 per litre of its own species. Mean inter-
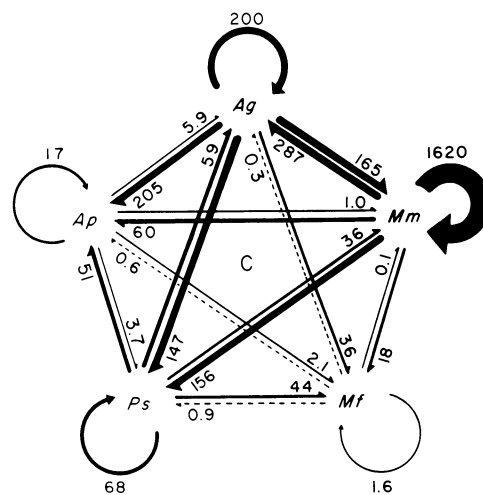
Fig. 1. Mean crowding per litre, upon which experimental densities were based in 13 holes at Blacknest 28 March – 6 April 1986. The *number* on the *straight arrow* indicates the mean crowding by the distant species on the species the arrow points to. *Thickness of lines* reflects relative values. For *circular arrows*, the value indicates mean intraspecific crowding. *Ag, Aedes geniculatus; Ap, Anopheles plumbeus; Mm, Metriocnemus martinii; Ps, Prionocyphon serricornis; Mf, Myiatropa florea*

specific crowdings were highest among interactions involving these same two species, exceeding 100/1 five times: *Metriocnemus* on *Aedes* and *Prionocyphon* and *Aedes* on *Anopheles, Prionocyphon,* and *Metriocnemus.* The remaining interspecific crowdings were 68/1 or less. The lowest mean intraspecific crowding was 1.6/1 of *Myiatropa florea* whose mean interspecific crowdings per litre on the other species were all 0.9/1 or less. No *D. dufouri* were found, even though Kitching (1971) found winter and early spring to be their period of greatest larval abundance.

Among the 11 holes at Blacknest where limnetic and benthic samples were maintained separately, 64% of the total organisms present were in the limnetic zone and 36% in the benthic (Fig. 2). Over 90% of the *Aedes geniculatus* were present in the limnetic zone while *Metriocnemus martinii* and *P. serricornis* were more evenly distributed between the zones. *Anopheles plumbeus* were found exclusively in the limnetic and *Myiatropa florea* exclusively in the benthic zones.

Species interactions – Aedes and Anopheles (Experiment 1)

Aedes: Pupation success of *A. geniculatus* in the spring and early summer at a density of 291/1 (Fig. 3) did not differ from pupation success of *A. geniculatus* at the same density but in the added presence of 45 *Anopheles plumbeus*/1 ($F_{1,6} = 1.09$; $P = 0.337$). Two-way ANOVA of pupal weight (Fig. 4A) showed that females were heavier than males ($F_{1,12} = 64.31$; $P < 0.001$) but neither the presence/absence of *Anopheles plumbues* ($F_{1,12} = 0.64$; $P = 0.768$) nor the interaction between sex and presence/absence of *A. plumbeus* ($F_{2,12} = 0.93$; $P = 0.414$) had a significant effect on pupal weight. In two holes that, by design, should have been occupied exclusively by *A. plumbeus,* some *Aedes geniculatus* escaped the censuses and produced pupae of both sexes. After log-transformation, 2-way ANOVA of *Aedes* pupal weights, including *Aedes* in the "*Anopheles plumbeus* only" holes, now revealed significant differences due to main treatments, ($F_{2,14} = 13.00$; $P = 0.0013$) as well as sex ($F_{1,14} = 88.73$; $P < 0.001$) but not their interaction ($F_{2,14} = 0.75$; $P = 0.495$). Duncan's Multiple Range Test ($P < 0.05$) indicated that pupal weights of *Aedes geniculatus* accidentally occupying holes with *Anopheles plumbeus* were heavier than in the other two treatments so that *Aedes geniculatus* developing at low densities, even in the presence of *Anopheles plumbeus,* were capable of achieving higher pupal weights. Two-way ANOVA of development time (Fig. 4B) showed that males developed faster than females ($F_{1,12} = 12.22$; $P = 0.004$) but development time was not affected by the presence/absence of *A. plumbeus* ($F_{1,12} = 0.36$; $P = 0.949$) or its interaction with sex ($F_{1,12} = 0.07$; $P > 0.999$).

Anopheles: Pupation success of *A. plumbeus* (Fig. 3) at a density of 45/1 did not differ from pupation success of *A. plumbeus* at the same density but in the added presence of 291 *Aedes geniculatus* per litre ($F_{1,6} = 0.08$; $P = 0.787$). Two-way ANOVA of pupal weight (Fig. 4C)
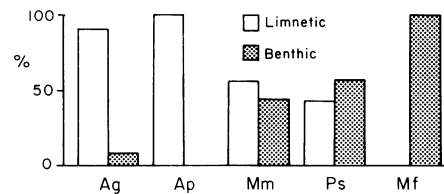

Fig. 2. Distribution of each insect species between limnetic (siphonable water) and benthic zones in 13 holes at Blacknest, 28 March – 6 April 1986. Abbreviations as in Fig. 1
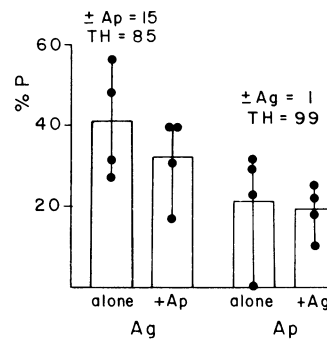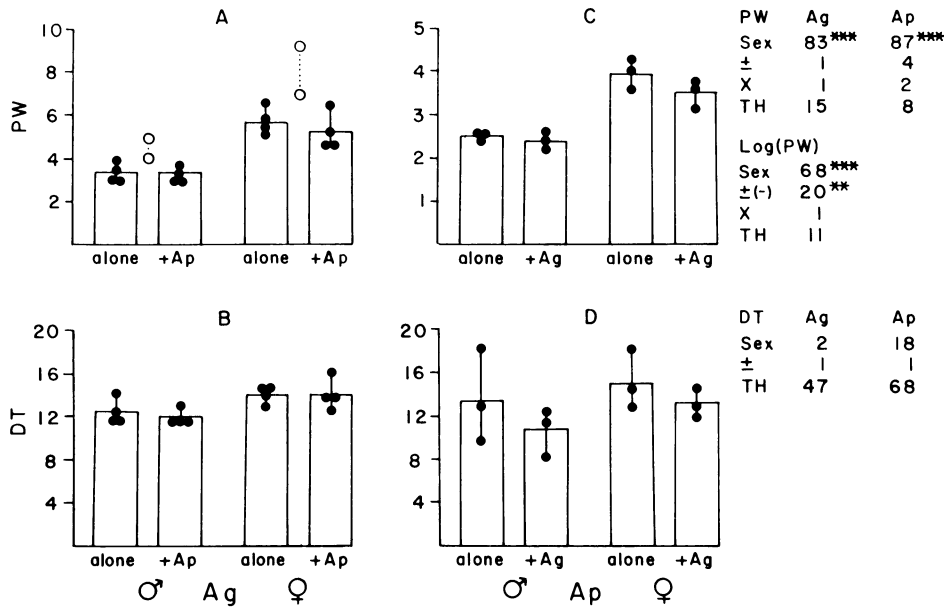

Fig. 3. Pupation success (%P, arcsine transformed) of *Aedes geniculatus* (*Ag*) and *Anopheles plumbeus* (*Ap*) when developing alone or in the added presence of the other species ($+ Ag$ or $+ Ap$) during the spring and early summer, 1986, at Silwood Park. *Bars* are means from four holes, each represented by a *dot.* The *tables* within the figure provide percent reduction in total sum of squares from the ANOVA attributable to the presence or absence of the other species ($\pm$) and residual variation among tree holes within treatments (*TH*). Thus, the presence or absence of *A. plumbeus* ($\pm Ap$) accounted for 15% of the variation in pupation success of *Aedes geniculatus* while differences among tree holes within treatments accounted for the remaining 85%

showed that female pupae were heavier than male pupae ($F_{1,8} = 87.39$; $P < 0.001$) but that there was no significant effect of the presence or absence of *A. geniculatus* ($F_{1,8} = 3.79$; $P = 0.087$) and no interaction effect ($F_{1,8} = 1.51$; $P = 0.337$). Two-way ANOVA also showed that development time of *Anopheles plumbeus* (Fig. 4D) was unaffected by sex ($F_{1,8} = 2.08$; $P = 0.148$), presence/absence of *Aedes geniculatus* ($F_{1,8} = 1.49$; $P = 0.251$), or their interaction ($F_{1,8} = 0.15$; $P = 0.995$).

Species interactions – Aedes and Culex (Experiment 2)

Survivorship, pupation success, and yield of both *Aedes geniculatus* and *C. torrentium* were measured by the same criteria. Consequently, we performed 2-way ANOVAs of survivorship, pupation success, and yield with treatments, species *(Aedes/Culex),* co-inhabitant of the tree hole (alone/together), and their interaction (Fig. 5). Survivorship (Fig. 5B) did not differ between *A. geniculatus* and *C. torrentium* ($F_{1,12} = 1.29$; $P = 0.278$), between developing alone or together with the other species ($F_{1,12} = 0.34$; $P = 0.958$), or their interaction ($F_{1,12} = 0.43$; $P = 0.929$). Similarly, pupation success (Fig. 5A) did not differ between *A. geniculatus* and *C. torrentium* ($F_{1,12} = 0.02$;

232

PW	Ag	Ap
Sex	83***	87***
±	1	4
X	1	2
TH	15	8

Log(PW)		
Sex	68***	
±(-)	20**	
X	1	
TH	11	

DT	Ag	Ap
Sex	2	18
±	1	1
TH	47	68

σ Ag ♀   σ Ap ♀

Fig. 4. Mean pupal weight (*PW*) and development time (*DT*) of *Aedes geniculatus* and *Anopheles plumbeus* developing alone or in the added presence of the other species (+ *Ag* or + *Ap*) during the spring and early summer, 1986, at Silwood Park. The *tables* to the right of the graphs provide percent reduction in total sum of squares from the ANOVA attributable to male-female differences *(Sex)*, the presence or absence of the other species (±), their interaction (*X*), or residual variation among tree holes within treatments (*TH*).

The *open circles* in the plot of *Aedes geniculatus* pupal weight show mean pupal weight for two tree holes that by design contained *Anopheles plumbeus* only but by accident produced a few *Aedes geniculatus*; the table to the right labeled *Log(PW)* provides percent reduction in total sum of squares from the ANOVA of log-transformed *A. geniculatus* pupal weight with three treatments by adding pupal weights from the unintentionally *A. geniculatus*-positive holes to the ANOVA immediately above it


Ag/Ct	=	1
±	=	1
X	=	1
TH	=	98

Ag/Ct	=	9
±	=	2
X	=	3
TH	=	86

Ag/Ct	=	2
±	=	1
X	=	3
TH	=	93
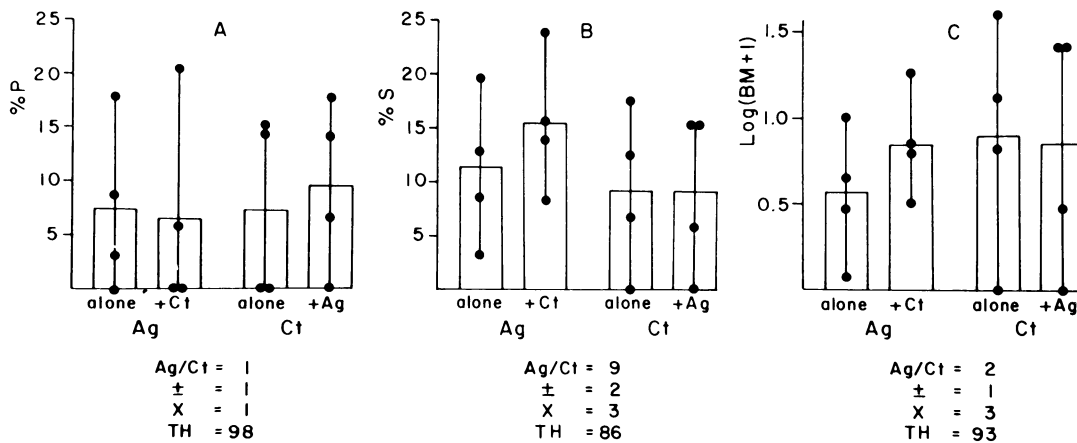
Fig. 5. Pupation success (%*P*, arcsine transformed), survivorship (%*S*, arcsine transformed), and biomass yield [Log(*BM* + 1)] of *A. geniculatus* and *C. torrentium* during the summer and fall, 1986, at Silwood park when developing alone *(alone)* or in the replacement presence of the other species (+ *Ag* or + *Ct*). The *tables* below each

graph provide percent reduction in total sum of squares from the ANOVA attributable to species (*Ag* or *Ct*), to the absence or replaced presence of the other species (±), to their interaction (*X*), or to residual variation among tree holes within treatments (*TH*)

P > 0.999), between presence/absence of the other species ($F_{1,12} = 0.13$; *P* > 0.999), or their interaction ($F_{1,12} = 0.12$; *P* > 0.999). Finally, biomass yield (Fig. 5C) did not differ between *A. geniculatus* and *C. torrentium* ($F_{1,12} = 0.31$; *P* > 0.983), between whether each species developed alone or together in the tree hole ($F_{1,12} = 0.16$; *P* = 0.999), or their interaction ($F_{1,12} = 0.43$; *P* = 0.944). Pupal weights were analyzed by species.

Aedes: Among *A. geniculatus* (Fig. 6A), female pupae emerged from only one hole so that a comparison of female pupal weights between treatments was not possible. Males emerged from five holes, three containing *A. geniculatus* alone, and two containing both *A. geniculatus* and *C. torrentium*. One-way ANOVA revealed no significant difference between mean male pupal weights due to the presence or absence of *C. torrentium*
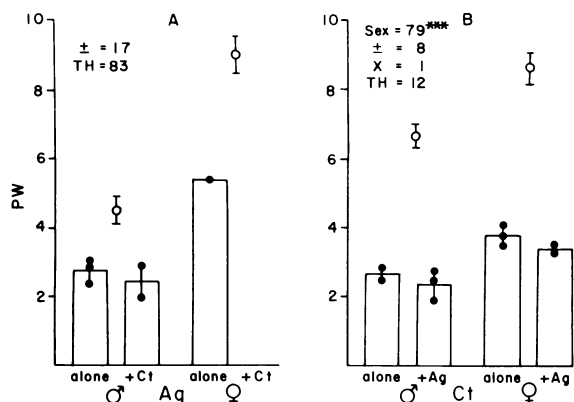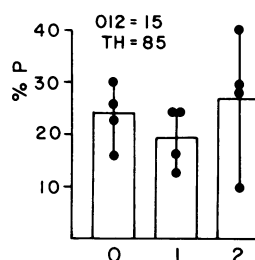
Fig. 6A, B. Mean pupal weight (PW) of *A. geniculatus* and *C. torrentium* during summer and fall, 1986, at Silwood Park when developing alone *(alone)* or in the replacement presence of the other species ($+Ag$ or $+Ct$). The *tables* within each graph provide the percent reduction in total sum of squares attributable to male-female differences *(Sex)*, presence or absence of the other species ($\pm$), their interaction, or residual variation among tree holes within treatments (TH). The *open circle* provides mean pupal weight $\pm$ exact 95% confidence limits observed **A** during the spring of *A. geniculatus* at reduced densities in 2 experimental holes at Silwood Park that by experimental design were to have contained *Anopheles plumbeus* alone (Exp. 1) or **B** of *C. torrentium* at reduced densities in a single tree hole at High Standing Hill

Fig. 7. Pupation success ($\%P$, arcsine transformed) of *A. geniculatus* at Blacknest in tree holes containing *A. geniculatus* plus no other insects (*0*), the normal density of benthic insects (*1*), or twice the normal density of benthic insects (*2*) during the spring of 1986. The *table* within the figure provides percent reduction in total sum of squares attributable to density of accompanying benthic insects (*012*) or to residual variation among tree holes within densities (*TH*)

($F_{1,3} = 0.62$; $P = 0.415$). Mean pupal weight from all five holes fell well below the 95% confidence limits of male or female pupal weights observed at lower density earlier in the season (see Experiment 1, above).

Culex: Mean pupal weights of female *C. torrentium* (Fig. 6B) were greater than those of males ($F_{1,6} = 35.83$; $P = 0.001$) but pupal weights were unaffected by the presence/absence of *A. geniculatus* ($F_{1,6} = 3.38$; $P = 0.173$), or their interaction ($F_{1,6} = 0.06$; $P = 0.998$). One tree hole (#17) at High Standing Hill contained overwintering *C. torrentium* at a lower density (2.5/l) than in the present experiment (300/l). Mean pupal weights of *C. torrentium* in the present experiment fell well below the 95% confidence limits for the pupal weight of overwintering *C. torrentium* at High Standing Hill (Fig. 6B). Thus, *C. torrentium* in nature were capable of achieving higher pupal weights than they did in the present experiment.

Species interactions – Aedes and benthic insects (Experiment 3)

In the spring and early summer, pupation success (Fig. 7) of *A. geniculatus* was not significantly affected by the absence, normal crowding, or super-abundance of *M. martinii* and *P. serricornis* ($F_{2,9} = 0.78$; $P = 0.487$). Two-way ANOVA of log(mean pupal weight) (Fig. 8A) showed that females achieved higher weights than males ($F_{1,17} = 77.59$; $P < 0.001$) but there was no direct effect of benthic insects ($F_{2,17} = 0.55$; $P = 0.587$) or interactive effect with sex ($F_{2,17} = 0.95$; $P = 0.406$). Two-way ANOVA of development time (Fig. 8B) also showed that females
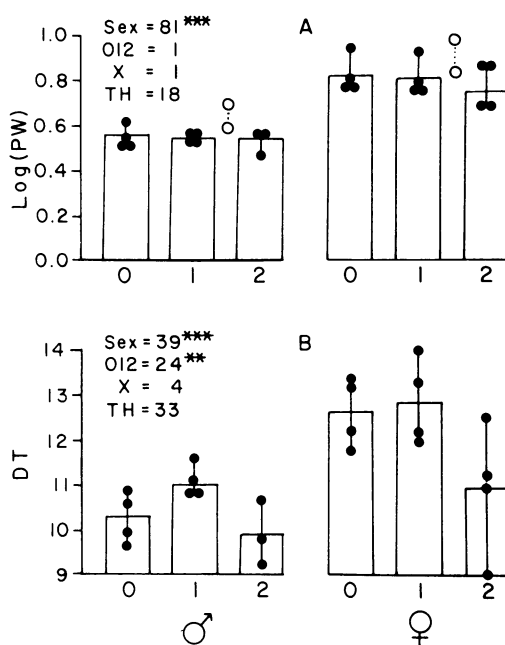
Fig. 8. Mean pupal weight (PW, log transformed) and development time (DT) of *A. geniculatus* in tree holes at Blacknest containing *A. geniculatus* plus no other insects (*0*), the normal density of benthic insects (*1*), or twice the normal density of benthic insects (*2*) during the spring of 1986. The *tables* within the figure provides percent reduction in total sum of squares attributable to male-female differences *(Sex)*, density of accompanying benthic insects (*012*), their interaction (*X*), or residual variation among tree holes within densities (*TH*)

developed more slowly than males ($F_{1,17} = 19.90$; $P < 0.001$) and that development time was affected by the density of benthic insects ($F_{2,17} = 5.97$; $P < 0.05$) but not sex-density interaction ($F_{2,17} = 0.95$; $P > 0.05$). Duncan's Multiple Range Test revealed that *A. geniculatus* developed faster when sharing tree holes with a superabundance than with a normal density of benthic insects but development time did not differ between the absence of benthic insects and their normal density or superabundance.

Discussion

Species interactions

Aedes and Anopheles: The addition of 45 *Anopheles plumbeus* per litre to 291 *Aedes geniculatus* per litre had no significant effect on pupation success, mean pupal weight, or development time of *A. geniculatus* (Figs. 3 and 4). Similarly, the addition of 291 *A. geniculatus* per litre to 45 *Anopheles plumbeus* per litre had no significant effect on pupation success, mean pupal weight, or development time of *A. plumbeus* (Figs. 3 and 4).

In eastern North America, *Aedes triseriatus* (Say) realize lower survivorship when holes (Copeland 1987) or experimental microcosms (Livdahl 1982) are also occupied by facultatively carnivorous *Anopheles barberi* Coquillett. When older instars of *A. barberi* encounter first instars of *Aedes triseriatus,* many of the latter are killed and eaten. Increased mortality is accompanied by increased per capita resources for the remaining larvae and, consequently, they pupate at higher weight (Livdahl 1982). Thus, when *A. triseriatus* co-occupy holes with *Anopheles barberi,* consequent decline in pupation success may be compensated for by an increase in subsequent adult fecundity resulting from the surviving larvae.

In the present case, the addition of *Anopheles plumbeus* to *Aedes geniculatus* did result in a non-significant decline in pupation success (Fig. 3) but also a non-significant decline, not rise, in mean female pupal weights (Fig. 4A). Thus, if *Anopheles plumbeus* has a slight competitive effect on *Aedes geniculatus,* these effects are more likely due to competition for resources than to an interference mechanism such as observed in analogous North American tree-hole mosquito communities.

By accident low densities of *Aedes geniculatus* occurred when some *A. geniculatus* remained or hatched into two of the holes designed to be occupied by *Anopheles plumbeus* alone. In this case, pupal weights of *Aedes geniculatus* were significantly heavier than those developing in holes at normal densities with or without *Anopheles plumbeus* (Fig. 4A) and approached the pupal weights of *Aedes geniculatus* females obtained under ideal conditions in the laboratory (Bradshaw and Holzapfel 1986). This result supports the earlier conclusion (Bradshaw and Holzapfel 1986) that the lower pupal weights observed in the field reflected density-dependent limitations to growth and development in native tree holes.

Aedes and Culex: Whether developing alone or together, *Aedes geniculatus* and *C. torrentium* did not differ in survivorship or pupation success (Fig. 5A, B). Neither species exhibited lower pupal weight (Fig. 6) or yield (Fig. 5C) in inter- than intraspecific encounters. Weights of available pupae were, however, lower than expected from natural tree holes where each developed at a lower density (Fig. 6). Thus, both *A. geniculatus* and *C. torrentium* experienced limiting resources but exhibited no competitive hierarchy even when they were forced to encounter each other in manipulated tree holes to a greater ($Z_{xy} = 200/1$) extent than they would under natural circumstances (Bradshaw and Holzapfel 1991: $Z_{AeCu} = 106/1$; $Z_{CuAe} = 22/1$).

In Britain, *C. torrentium* is normally abundant during the mid- to late summer, regardless of whether or not they occupy tree holes (Onyeka 1980). Even if *A. geniculatus* and *C. torrentium* compete evenly for limiting resources, temporal habitat segregation between these two benthic-limnetic filter feeders and browsers means that each species encounters primarily others of its own kind. Consequently, intraspecific competition exceeds interspecific competition and these two species should continue to coexist.

Culex and Anopheles: *Anopheles plumbeus* was the least abundant mosquito in the community; its population was never as high as *Aedes geniculatus* or *C. torrentium* (Bradshaw and Holzapfel 1991), but persisted throughout the year, encountering more of other mosquito species than its own. Interspecific encounter by *Anopheles plumbeus* was dominated by *Aedes geniculatus* in the spring and by *C. torrentium* in the summer (Bradshaw and Holzapfel 1991). *Anopheles plumbeus* shows a degree of macro-habitat segregation from *C. torrentium* by occupying more cryptic holes (Bradshaw and Holzapfel 1986) and micro-habitat segregation based on their surface feeding habits. No direct manipulations were made to test for species interactions between *A. plumbeus* and *C. torrentium;* but, given the lack of a significant effect of large numbers of *Aedes geniculatus* on *Anopheles plumbeus* performance (Figs. 3 and 4) and both macro- and microhabitat segregation between *A. plumbeus* and *C. torrentium,* it is likely that they, too, interact only weakly.

Aedes and the benthic fauna: The non-mosquito benthic fauna consisted primarily of *Metriocnemus martinii* and *Prionocyphon serricornis* but also a few *Myiatropa florea* (Fig. 2). The former two species had little impact on performance of *Aedes geniculatus* (Figs. 7 and 8) regardless of their absence or their presence at normal or twice normal densities. The only significant effect of the benthic insects on *A. geniculatus* was accelerated, not retarded, development when the benthic insects were present at abnormally high densities. Part of the basis for a lack of significant interaction may come from microhabitat segregation between the benthic and limnetic zones (Fig. 2). However, it is also possible that the benthic and mosquito faunas do consume common resources but that consumption competition (sensu Schoener 1983) is compensated for by recycling of deep detrital nutrients through physical activity of the benthic fauna. Evidence for the latter interpretation comes from the observation that the only significant effect of the benthic fauna on *A. geniculatus* is facilitative.

Regardless of the underlying mechanisms of interaction, it is clear from Fig. 7 and 8 that variation in tree-hole quality greatly exceeds any net effect of the benthic fauna on *A. geniculatus,* even at densities where the latter are resource-limited. Consequently, the mosquito and benthic faunas should be able to continue coexistence indefinitely.

Bases and implications of coexistence

In the case of *Aedes geniculatus* (Figs. 4A, 6A; Bradshaw and Holzapfel 1986), *Anopheles plumbeus* (Bradshaw and Holzapfel 1986), and *C. torrentium* (Fig. 6B), individuals achieve higher pupal weights at unusually low densities in nature or in the laboratory than they do at normal densities in tree holes. Even when species in the present study were forced to coexist at abnormally high levels of interspecific crowding, they did not adversely affect the performance of one another. Indeed, the only significant interaction is positive (Fig. 8). We therefore conclude that intraspecific competition exceeds adverse interspecific interaction and that the mosquito fauna should be able to coexist indefinitely with each other and with the benthic fauna.

The ultimate causes for habitat segregation are not obvious. On the one hand, competition, competitive exclusion, and competitively driven niche shifts can be invoked to explain habitat segregation and consequent coexistence (Cody 1968; Cody and Diamond 1975; MacArthur and Levins 1967; Schoener 1974). On the other hand, habitat segregation may have evolved independently and incidentally allowed coexistence when the various species first encountered one another. Evidence for the latter scenario emerges from comparing the components of habitat segregation of British tree-hole mosquitoes with other species in their own respective genera:

Anopheles as a genus are specialized surface feeders whether or not they exploit tree holes or coexist with other species. Among tree-hole anophelines, those in the Nearctic (Copeland and Craig 1990b; Zavortink 1970; Bradshaw and Holzapfel 1988) as well as the Palearctic (Bradshaw and Holzapfel 1986) exploit deeper, more permanent tree holes similar to the rot holes at Silwood Park. If anything, the occurrence of *A. plumbeus* in the basal pan holes represents an expansion of *Anopheles* habitat usage when compared with the North American species.

Aedes as a genus are limnetic-benthic filter feeders with drought-resistant eggs. In both western (Hawley 1985a) and eastern (Bradshaw and Holzapfel 1983, 1984; Shroyer and Craig 1981; Copeland 1987) Nearctic tree-hole *Aedes*, larval population densities are highest during vernal recruitment and decline as the season advances. This pattern persists regardless of whether they are living in a southern or northern climate, in the presence or absence of *Anopheles*, or in the presence or absence of major predators. Thus, the temporal pattern of tree-hole utilization by *A. geniculatus* is characteristic of tree-hole *Aedes* in general; it probably has little to do with competition from *Anopheles plumbeus* and certainly is not related to competition with *C. torrentium* since the seasonal pattern of vernal prevalence and subsequent decline of *A. geniculatus* existed in Britain (Kitching 1971; Yates 1979) when it did not encounter *C. torrentium*.

C. torrentium is a closely related, sibling species to *C. pipiens* L. (Service 1968). Both species oviposit into small ponds, puddles, ditches, artificial containers, and even brackish water (Service 1968) but only *C. torrentium*

oviposits into tree holes and both show the same seasonal pattern of development (Onyeka 1980). Thus, temporal habitat specificity of *C. torrentium* reflects that of non-tree-hole individuals of the same species and of species in their immediate ancestry. Summer habitat specificity of *C. torrentium* has therefore not evolved as a consequence of coexistence with *A. geniculatus*.

The above discussion supports the contention that British tree-hole mosquitoes partition their usage of tree holes in much the same way as their congeneric species living elsewhere and in other habitats. Consequently, habitat segregation has more likely evolved independently of their encounter with one another than as a consequence of recent competition in British tree holes. Indeed, the segregation of the three mosquito species among seasons and tree-hole types (Bradshaw and Holzapfel 1986, 1991) may be sufficient but not necessary for coexistence since we could not demonstrate significant or substantial interaction even when we experimentally forced these species to overlap in space and time.

Invasion of tree holes by Culex torrentium

Culex torrentium is a relatively new immigrant into Britain (Mattingly 1951; Service 1968). Service (1968) successfully collected egg rafts in bamboo pots but *C. torrentium* were not actually observed in tree holes until 1978 by Onyeka (1980). Earlier studies on tree-hole fauna in Britain (Beattie and Howland 1929; Blacklock and Carter 1920; Keilin 1927; Yates 1979) all concerned rot holes where *C. torrentium* is rare or absent (Bradshaw and Holzapfel 1986). Thus it is not until Kitching's (1971) study during 1967–1968 at Wytham Woods near Oxford that people were looking in the correct types of tree holes. Kitching (1971) found no *C. torrentium* in his 2-year-long, bi-monthly censuses of 30 beech buttress holes. Onyeka (1980) examined 17 tree holes in the southeast corner of Silwood Park (primary study area in the present investigation) "during the summers of 1978 and 1979" and found a total of 73 *C. torrentium*. In August 1984, 503 *C. torrentium* were found in 17 beech buttress holes at Silwood Park and High Standing Hill combined (Bradshaw and Holzapfel 1986). By August 1986, *C. torrentium* was the most prevalent tree-hole mosquito at Silwood Park (Bradshaw and Holzapfel 1991). Bradshaw and Holzapfel (1986) found *C. torrentium* restricted to the largest exposed buttress holes in beech trees; by 1986 (Bradshaw and Holzapfel 1991), they had extended their range into cryptic buttress holes (in beech trees) as well.

Culex torrentium is invading a new habitat at a place and time where resources are available to them. The degree to which *C. torrentium* encounter interspecific competition in other habitats is not known. Niche expansion in tree holes from exposed to cryptic buttress holes is, if anything, being made in the direction of increased interspecific encounter since the cryptic buttress holes are more likely to contain *Anopheles plumbeus* (Bradshaw and Holzapfel 1986). Competition in tree holes cannot be invoked as a reason for spatial and temporal occurrence of *C. torrentium* or its niche shift within tree holes.

Elsewhere, assemblages of tree-hole mosquitoes are far more diverse. Lu et al. (1980) found 32 sympatric species of tree-hole mosquitoes in mountain forests of Hainan Island. Lounibos (1981) described habitat segregation among 22 sympatric species in tropical Kenya; modes of macro-habitat segregation were broader and micro-habitat segregation more refined than in either the present study in Britain or in eastern North America (Bradshaw and Holzapfel 1983, 1988; Copeland 1987). It has been proposed earlier (Bradshaw and Holzapfel 1983) that the number of mosquito species in tree-hole assemblages may depend upon both the richness of the surrounding mosquito fauna and the number of other available container habitats. Such "stepping stone" habitats are rare in western Europe. Interestingly, *C. torrentium* is more prevalent in artificial containers than is *C. pipiens* (Onyeka 1980) and it may be that human artifacts and trash have provided the stepping stones to tree holes.

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