

Fitness and habitat segregation of British tree-hole mosquitoes

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Abstract. 1. Populations of three mosquitoes, *Aedes geniculatus* (Olivier), *Anopheles plumbeus* Stephens and *Culex torrentium* Martini, were found in three types of tree holes: rot holes penetrating the bark of the host tree and exposed, or deeper, more cryptic, bark-lined buttress holes. *Aedes geniculatus* occupied all three tree-hole types but reached their greatest abundance in exposed buttress holes; *A. plumbeus* predominated in rot holes; *C. torrentium* occupied buttress holes exclusively.

2. After a 1 month period of winter freezing, larvae of *A. plumbeus* survived better than larvae of *A. geniculatus*. Freeze resistance of *A. plumbeus* increased from cryptic buttress to rot holes. Freeze resistance of *A. geniculatus* declined from exposed buttress to cryptic buttress to rot holes and was markedly higher in more protected than in more exposed macrohabitats.

3. Among manipulated larval cohorts of *A. geniculatus* and *A. plumbeus*, survivorship, pupation success, pupal weight, and/or biomass yield did not differ among tree-hole types or was the reverse of freeze-related survivorship. Despite seasonal differences in fitness correlates, annual fitness may be similar among tree-hole types for both species. Manipulations carried out at a single census or during a single season are therefore likely to produce misleading information about fitness variation among sub-habitats.

4. Both species should exploit a broad range of tree-hole types but *A. plumbeus* occupies more restricted types of tree holes than does *A. geniculatus*. The restricted habitat usage by *A. plumbeus* does not parallel their fitness among tree-hole types in southern Britain but does resemble that of other tree-hole anophelenes. We therefore propose that habitat selection by *A. plumbeus* is more likely due to their phylogenetically determined physiological tolerances and behaviour than to competition from coexisting *A. geniculatus*.

Key words. Seasonality, cold-hardiness, recruitment, habitat-choice.

Introduction

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The struggle for existence, a fundamental basis
for Darwinian selection, implies that species

compete for resources both among themselves and with other species. Consequently, whenever two species share limiting resources, the conventional view is that either one must go extinct or one or both species must evolve sub-habitat specializations. In the latter case, each species then becomes limited more by members of its own than the other species. The two species may then continue to coexist indefinitely in the broader habitat by exploiting distinct sub-divisions of that habitat (Cody, 1968; MacArthur & Levins, 1967; Schoener, 1974; Pianka, 1969; Cody & Diamond, 1975). The classic examples of habitat segregation occur among animals that are able to modify their habitat utilization behaviourally over wide spaces. Examples have since proliferated and hundreds now exist (Schoener, 1974, 1986).

Intuition would suggest that the potential for competition should increase in more limited spaces and the degree of habitat segregation should also increase. Hutchinson's (1959) 'Homage to Santa Rosalia' considered the coexistence of but two species of corixid bugs in a small limestone spring, not the fifteen or more species that may occur in larger ponds (Macan, 1962; Walton, 1943). Highly circumscribed habitats frequently occur in plant-held waters (phytotelmata) such as pitcher-plant leaves, bromeliad axils and tree holes (Frank & Lounibos, 1983). Of these habitats, tree holes have the widest distribution and are the most widely studied. Throughout the world, the most frequent, consistent inhabitants of tree holes are mosquitoes that may exhibit spectacular habitat segregation within as well as between tree holes (Lounibos, 1981; Bradshaw & Holzapfel, 1983, 1985, 1986, 1988).

Tree holes in southern Britain may contain *Aedes geniculatus*, *Anopheles plumbeus*, *Culex torrentium* and/or *Orthopodomyia pulchripalpis* (Marshall, 1938; Fallis & Snow, 1983; Keilin, 1927; Yates, 1979; Bradshaw & Holzapfel, 1986). These four species exhibit distinct habitat segregation (Bradshaw & Holzapfel, 1986). *O. pulchripalpis* occupies large, deep, permanent rot holes that are found in very old or pollarded beech and plane trees. *Orthopodomyia pulchripalpis* is now rare in Britain and northwestern Europe, probably due to habitat reduction by human activity. *Anopheles plumbeus* may co-occur with *O. pulchripalpis* but also extends habitat usage into smaller rot

holes and deeper, more cryptic buttress holes (= pans, *sensu* Kitching, 1971) in beech trees. *Aedes geniculatus* occupies the smaller rot holes and cryptic buttress holes along with *A. plumbeus* but also abounds in the largest, most exposed buttress holes as well. *C. 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.

A basic assumption underlying studies of habitat segregation is that fitness varies with habitat utilization. That is, species specialize in that subhabitat in which they realize the greatest relative fitness. In the present study, we examine this assumption by asking whether fitness realized by two of the British species, *A. geniculatus* and *A. plumbeus*, varies according to the type of hole they occupy.

Materials and Methods

Experimental rationale. Several components of mosquito development are correlated with fitness and provide measures of overcrowding or of resource limitations. Low per capita resources result in retarded larval growth and development, reduced pupal weight, reduced survivorship, reduced pupation success and reduced biomass yield (Hawley, 1985a, b; Hard *et al.*, 1989; Fisher *et al.*, 1990; references therein). These components of development were formally defined as follows:

Pupation success = cumulative number of pupae recovered from each hole throughout the experimental period divided by the number of larvae in the original cohort.

Pupal weight = mean pupal weight of each sex of all pupae recovered from each hole during the experimental period.

Development time = mean weeks to pupation of each sex among all pupae recovered from each hole during the experimental period.

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 *A. geniculatus*

or number of third and fourth instars in the original cohort for *A. plumbeus*.

Yield = (cumulative sum of pupal weight regardless of sex plus the sum of larval weight 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 removed in the field, identified, sexed, and weighed to the nearest 0.1 mg on a microbalance. 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) and using a dry-wet weight conversion factor determined for *Aedes sierrensis* in the laboratory:

wet weight (mg) = 0.25 + 4.84dry weight (mg)

Sampling localities. All of the observations and experiments were performed at the 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). At all localities, woodlands consisted primarily of c. 200–350-year-old beech trees (*Fagus sylvatica* L.) mixed with younger beech, oak (*Quercus petraea* (Mattuschka) Liebl. and *Q. robur* L.), chestnut (*Aesculus hippocastaneum* L. and *Castanea sativa* Mill.), birch (*Betula pendula* Roth) and maple (*Acer pseudoplatanus* L.). The understorey was comprised mainly of holly (*Ilex aquifolium* L.), rhododendron (*Rhododendron ponticum* L.) and bracken (*Pteridium aquilinum* (L.) Kuhn).

Silwood Park lies on rolling countryside. The Blacknest area extends over level ground along the southern rim of Virginia Water, a large (c. 50 ha) man-made lake. The High Standing Hill locality consists of a northeast to southwest ridge c. 87 m in elevation with land sloping down to the south for a total drop of about 22 m within the study area. Trees along the ridge cover little understorey; those on the south-facing slope cover stands of rhododendron and are interspersed with stands of bracken fern.

Observations of the overwintering population were made at all three localities. Experimental manipulations were performed only at Silwood Park; populations at High Standing Hill provided an unmanipulated control.

Sampling technique. Tree holes were drained with a 2 cm inside diameter, clear Tygon siphon.

The siphon was always inserted into the deepest and/or darkest corner of the hole where the negatively phototropic larvae and pupae tended to concentrate. Aspiration began immediately upon insertion so as to capture *Anopheles* on the surface and all species in the water column before they escaped into the leaves and detritus at the base of the hole. The water was drained into buckets and the volume measured. Each hole was siphoned three times. Each siphoning was counted only if a rapid, smooth-running flow could be established; otherwise, the siphoning was repeated until three satisfactory siphonings had been achieved. Water for the second and third siphoning was obtained by straining each siphonate through a fine-mesh aquarium net and pouring the filtrate back into the most exposed, best lit portion of the hole to facilitate movement of mosquitoes to deeper, darker regions. After the third successful siphoning, larvae and pupae were concentrated into 0.95 litre plastic bags, transported to the laboratory, and stored at 4±2°C in a refrigerator until they were counted.

In the laboratory, samples were placed in a plastic pan that was nested into a second pan filled with ice. This technique maintained sample temperature at or below those prevailing in the field. Larvae and pupae were removed and numbers of each instar and each species recorded. Samples, complete except for pupae, were placed after censusing back into the incubator and returned to their respective holes on the same day as sampling.

Since the goal was to assess the effects of tree-hole type on mosquito fitness, water volume was maintained in all tree holes (including controls) at March 1986 levels by the addition of glass-distilled water after each census.

Density. Studies in predator-free tree-hole populations in western North America (Hawley, 1985a, b) had shown the larval stage to be limiting and previous observations on European tree-hole mosquitoes (Bradshaw & Holzapfel, 1986) were consistent with this conclusion. Consequently, all manipulations involved larval densities. The important density is that perceived by the average individual in the population, not the density in the average habitat. Consequently, all of the experimental densities were based on levels of mean crowding actually observed in 1986. Mean crowding of species *y* on species *x* per litre volume (*v*) in the *i*th tree

hole was calculated as (Lloyd, 1967; Hurlbert, 1978):

$$Z_{xy} = \Sigma[(x_i y_i) / v_i] / \Sigma x_i \quad (1)$$

and mean intraspecific crowding by substituting $(x_i - 1)$ for y in the above equation. Densities for spring experiments were based on mean crowdings observed in forty-two holes prior to manipulation at Silwood Park and at Blacknest. Densities for summer experiments were based on additional mean crowdings observed in thirty-seven 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 *A.geniculatus* occurred immediately and increased as temperatures rose during March. Forty tree holes at Silwood park were located and censused from 7 to 11 March and seventeen 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.

Statistical procedures Analysis of variance (ANOVA) and regression were performed according to procedures outlined in Sokal & Rohlf (1969). Frequencies of mortality and survivorship in the overwintering population considered community-wide patterns that were not part of a designed experiment. Consequently, the *G*-test of independence (log-likelihood ratio test) was used to analyse these data. All percentage values in pre-planned experiments were transformed by the arcsine transformation prior to regression or ANOVA. All ANOVA's 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 $\log(x+1)$ transformations rendered the variances non-heterogeneous. When two-way ANOVA involved unequal and disproportionate sample sizes, ANOVAs were performed using the SAS General Linear Model (SAS GLM) procedure (SAS Institute, 1985).

Experimental procedures. 1. *Fitness and tree-hole type: spring.* The purpose of this and the subsequent experiment was to assess the effect of macro-habitat (tree-hole type) on the fitness of *A.geniculatus* and *A.plumbeus*. *A.plumbeus* were relatively scarce so that the relative fitness of *A.geniculatus* alone was considered during the spring in rot, cryptic buttress, and exposed buttress holes at Silwood Park.

Mean intraspecific crowding of *A.geniculatus* equalled 337 larvae per litre (Fig. 1A) with 0.859 of the larvae in the first instar, 0.067 in the second, 0.036 in the third, and 0.038 in the fourth. Larvae from these censuses were not replaced but used to establish experimental populations. Experimental density was set at 337 per litre with the same proportion of instars as in the 22–28 March census in each of four rot, four cryptic buttress, and four exposed buttress holes. Larvae were pooled by instar and stored in a refrigerator until 31 March. On 31 March the experimental populations were counted out and placed in their prescribed tree holes that had been cleared of mosquito larvae during the preceding censuses.

Since hatching of *A.geniculatus* appeared to be a continuing process, experimental holes were censused 30 April to 5 May and again on 20–21 May. At each census, densities were readjusted to 337/litre by the removal of first instars or by the addition of first instars recently collected from remote, non-experimental holes at Silwood Park or Blacknest. No further adjustments were made after the May censuses by which time annual recruitment was largely complete (Table 1). The experimental populations were censused again 21 June, 15–16 July, and, finally, on 4–6 August when the experiment was terminated. Experiments were terminated at this time due to the dwindling number of remaining *Aedes*.

Upon the appearance of the first *Culex* egg rafts in mid-July, each tree hole was checked with a torch every 48–72 h and all egg rafts removed.

Fitness of *A.geniculatus* in each hole was estimated from pupation success, pupal weight and development time, as defined previously. Variation in pupation success was assessed by one-way ANOVA of arcsine transformed percentage pupation among the three tree-hole types (rot/cryptic buttress/exposed buttress). Variation in log (pupal weight) and develop-

ment time was assessed by two-way ANOVA with treatments sex (male/female) and tree-hole type (rot/cryptic/exposed).

2. *Fitness and tree-hole type: summer.* At the end of the previous experiment, sufficient *Anopheles plumbeus* were appearing in tree holes to permit a comparison of relative fitness of *Aedes* and *Anopheles* in rot and cryptic buttress holes. During the summer, appearance of *Aedes* larvae into holes had declined while that of *Anopheles* was increasing. Consequently, the design of this experiment was to introduce into holes first instar *Aedes* plus all of the available *Anopheles* and to assess their individual fitness in these two tree-hole types. After the start of the experiment, *A. plumbeus* but not *A. geniculatus* continued to appear in some of the holes; consequently, first and second instar *Anopheles* that appeared in weekly or biweekly censuses were presumed to be recruits and were redistributed among the holes on a per litre and per instar basis.

After censusing and removing all mosquito larvae from all holes, 300 first instar *A. geniculatus* per litre plus 13.3 *A. plumbeus* per litre were introduced into each of four rot and four cryptic buttress holes. The *Anopheles* were introduced at the then prevailing instar distribution: 0.10 first, 0.10 second, 0.30 third and 0.50 fourth. Thus, densities were, at the outset, slightly lower than the 337/litre used in the spring but allowance was made for the ongoing addition of further *Anopheles*. *Aedes* were obtained as the F₁ of the overwintering generation from a colony maintained in a 1 m³ cage in a humidified glasshouse at Silwood Park. *Anopheles* were obtained from non-experimental holes at Silwood Park and at Blacknest.

The experiment was started 8 August 1986. Censuses and redistributions of *Anopheles* were made on 22 and 27 August, 3, 10 and 23 September, and 7 October. Each tree hole was checked for *Culex* egg rafts and the rafts removed every 48–72 h throughout the experiment. Once each week, glass-distilled water was added to the holes to maintain the original volume and, at the same time, pupae were removed. Pupae were immediately transported back to the laboratory, identified, sexed and weighed. The experiment was terminated and the final census taken on 22 October 1986, when all pupation of *Aedes* and *Anopheles* had

ceased in the unmanipulated holes at High Standing Hill.

Both *A. geniculatus* and *A. plumbeus* may overwinter as larvae and fitness could be measured both by pupation success and, at the end of the experiment, by survivorship and by yield. Variation in pupation success and survivorship was assessed using one-way ANOVA of arcsine transformed pupation or survivorship among the two tree-hole types. Analysis of mean pupal weight differed between *A. geniculatus* and *A. plumbeus*. In *A. geniculatus*, too few holes produced female pupae for a meaningful comparison; mean male pupal weights were subjected to one-way ANOVA between tree-hole types. In *A. plumbeus*, individual holes did not always produce both male and female pupae; consequently, mean pupal weight was subjected to two-way ANOVA using the SAS GLM procedure with treatments sex, tree-hole type, and their interaction. Variation in log (biomass yield) for each species was assessed using one-way ANOVA of yield between tree-hole types.

Results

Overwintering survivorship

At Silwood Park, mean daily air temperatures ranged from 0 to +10°C in January, –7 to +3°C in February, and –1 to +10°C in March. Snow covered the ground from 6 February to 3 March. Tree holes were frozen during the same period of time that snow remained on the ground, began to thaw on 5 March, and were fully thawed on 7 March. Freshly hatched (untanned) first instars of *A. geniculatus* appeared on the first day of thawing at tree-hole temperatures of 0–3°C and, within a week, became indistinguishable from (tanned) first instars that had hatched before the freeze. Since first instars appeared to represent a minor component of the overwintering larval population, estimates of overwintering survivorship were based on second, third and fourth instars. No untanned larvae of *A. plumbeus* were observed until mid-summer; consequently estimates of overwintering survivorship in this species were based on all instars.

Survivorship of *Aedes geniculatus* depended upon locality, presence of *Anopheles plumbeus*, and tree-hole type (Fig. 2). A *G*-test for

Table 1. Presence on 7–15 March of second to fourth instars after the winter freeze and appearance of first instars thereafter in four rot (R), four cryptic buttress (C) and four exposed buttress (E) holes at Silwood Park in 1986.

Dates of census	Hole type	No. of <i>Aedes</i>	No. of <i>Anopheles</i>	No. of <i>Culex</i>
7–15 March	R	2	78	0
	C	67	5	0
	E	193	0	0
28 March	R	494	28	0
	C	674	0	0
	E	1022	0	0
20–21 May	R	58	0	0
	C	151	0	0
	E	171	0	0
17–21 June	R	14	0	0
	C	46	0	0
	E	7	1	0
15–16 July	R	1	49	4
	C	4	0	465
	E	5	0	114
4–6 August	R	25	6	7
	C	0	0	396
	E	8	2	631

independence of survivorship in forty holes at Silwood Park, seventeen at Blacknest, fifteen at High Standing Hill on the ridge, and ten at High Standing Hill on the south-facing, protected slope with and without *Anopheles plumbeus* revealed non-independence both for survivorship and locality (Fig. 2A) (G with 3 df = 788; $P < 0.001$) and for presence or absence of *A. plumbeus* (Fig. 2B) (G with 1 df = 4.30; $P < 0.05$). Survivorship was higher in holes on the south-facing slope at High Standing Hill and at Blacknest than at Silwood Park or on the ridge at High Standing Hill. Among all eighty-two holes, survivorship of *Aedes* was higher in holes it occupied with *Anopheles* than in those it occupied alone. At Silwood Park, survivorship of *Aedes* was dependent upon tree-hole type (Fig. 2C) (G with 2 df = 413; $P < 0.001$). Survivorship was highest in four exposed buttress holes, lower in eighteen cryptic buttress holes, and lowest in eight rot holes.

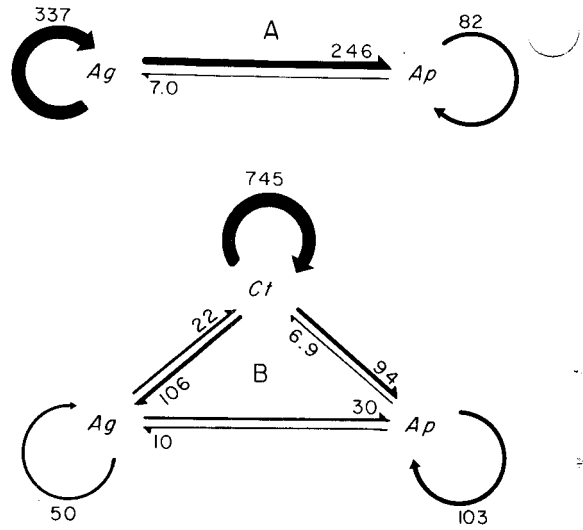


Fig. 1. Mean crowding per litre upon which experimental densities were based: (A) in forty-two holes at Silwood Park and Blacknest, sampled 22–28 March 1986; (B) in thirty-seven holes at High Standing Hill, sampled 21–27 July 1986. The number on the straight arrow indicates the mean crowding by the distant species on the species the arrow points to. Thickness of lines reflects relative values. For circular arrows, the value indicates mean intraspecific crowding.

Survivorship of *Anopheles plumbeus* also depended upon locality, presence of *Aedes geniculatus*, and tree-hole type (Fig. 2D–F). A G -test for independence of *Anopheles* survivorship revealed non-independence both for *Anopheles* survivorship and locality (Fig. 2D) (G with 3 df = 37.33; $P < 0.001$) and for *Anopheles* survivorship and presence of *Aedes* (Fig. 2E) (G with 1 df = 21.8; $P < 0.001$). *Anopheles* survivorship was highest at either site at High Standing Hill but lower at Silwood Park (Fig. 2D). Among all eighty-two holes, survivorship of *Anopheles* was higher in holes it occupied alone than in holes it occupied with *Aedes* (Fig. 2E). At Silwood Park, survivorship was dependent upon tree-hole type (G with 1 df = 15.57; $P < 0.001$) where *Anopheles* survived better in eight rot holes than in eighteen cryptic buttress holes (Fig. 2F). No *Anopheles* were found overwintering in the four exposed buttress holes.

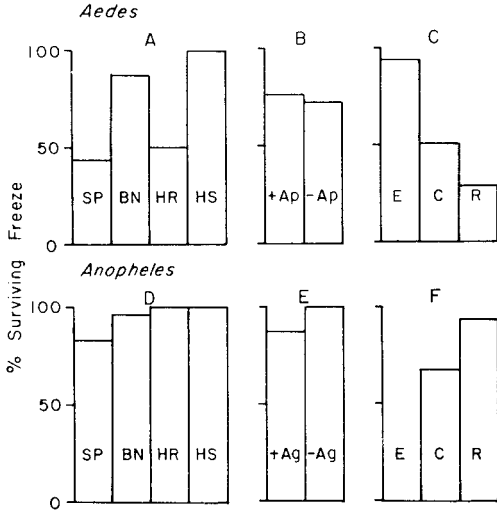


Fig. 2. Survivorship of *A. geniculatus* and *A. plumbeus* through a freezing period 6 February to March 1986, based on censuses of live and dead larvae found when holes subsequently thawed. The abbreviations within the histograms denote: SP, Silwood, Park; BN, Black-nest; HR, along an exposed ridge and HS, on a protected, south-facing slope at High Standing Hill. +An and -An, survivorship of *A. geniculatus* in holes with and without *A. plumbeus*, respectively; +Ae and -Ae, survivorship of *A. plumbeus* in holes with and without *A. geniculatus*, respectively. E, C, R, exposed buttress, cryptic buttress and rot holes.

Recruitment

Recruitment of mosquitoes during the year (Table 1) was estimated from censuses of the twelve holes (four rot, four cryptic buttress, four exposed buttress) used to evaluate fitness and tree-hole type (Experiment 1). Recruitment from overwintering larvae was equated with the survivors (second through fourth instars) found after the freeze in censuses from 7–15 March. *Culex torrentium* were absent; *Anopheles plumbeus* occurred in rot, and to a lesser extent, in cryptic buttress holes; *Aedes geniculatus* were found in all three tree-hole types, but mainly in the exposed buttress holes. Subsequent hatching was revealed by first instars on 28 March. Again, there were no *Culex*; a few *Anopheles* first instars occurred only in rot holes; *Aedes* hatched in large numbers into all hole types. The experiment to assess fitness and tree-hole type examined only *Aedes*; their numbers were set

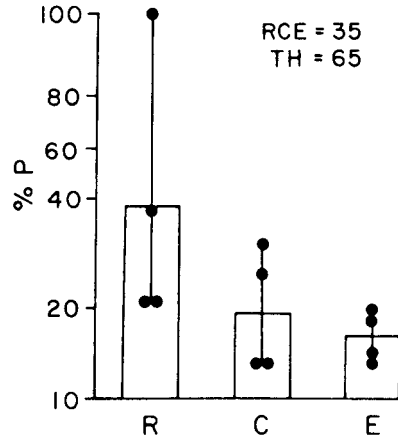


Fig. 3. Pupation success of *A. geniculatus* in rot (R), cryptic buttress (C) and exposed buttress (E) holes at Silwood Park during the spring of 1986. Solid circles correspond to individual tree holes. The inset in the figure provides percentage reduction in total sum of squares from the ANOVA attributable to tree-hole type (RCE) and variation among tree holes within tree-hole types (TH). Significance in this and subsequent ANOVA insets is given by: no $*P > 0.05$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. The vertical axis reflects the $\log_{10}[\sin^{-1}(\% \text{ pupation})^{1/2}]$ transform to achieve non-heterogeneous variance prior to ANOVA.

experimentally for the last time on 2–5 May. Subsequent hatching declined and remained low for the rest of the experiment. *Anopheles* first instars were absent or at very low levels through June, appeared in rot holes in July and August with one or two appearing in exposed buttress holes during June–August. No *Anopheles* appeared in cryptic buttress holes during the experiment. *Culex* were absent from all holes until July and August when they hatched in large numbers in cryptic and exposed buttress holes with only four to seven individuals appearing in rot holes.

These results show that *Aedes* occurred in all tree-hole types, with their greatest abundance due to first instars that hatched immediately following the freeze. *Anopheles* hatched mainly into rot holes during July, August, or later and then continued as overwintering larvae. *Culex* hatched primarily into cryptic and exposed buttress holes during July and August.

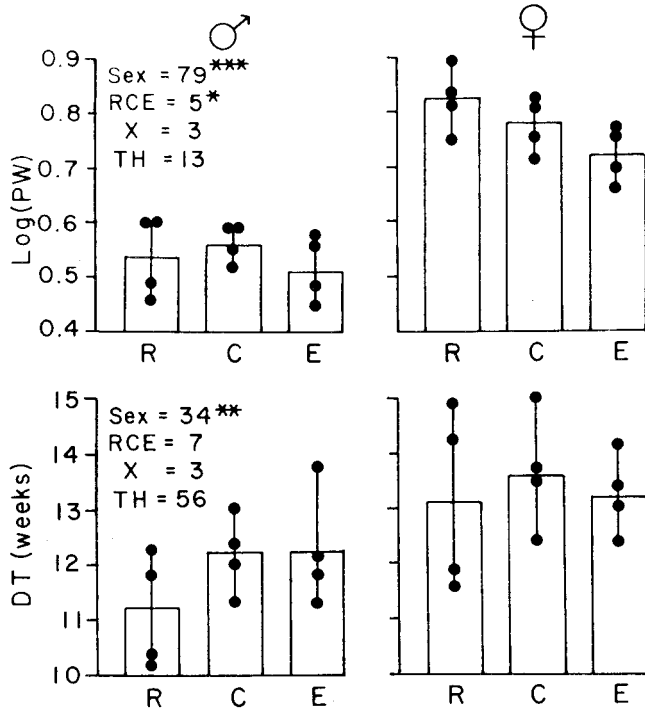


Fig. 4. Mean pupal weights (PW, in milligrams) and mean development times (DT, in weeks to pupation since start of the experiment) in rot (R), cryptic buttress (C) and exposed buttress (E) holes at Silwood Park during spring 1986. The tables within each graph provide percentage reduction in total sum of squares attributable to male–female differences (Sex), tree-hole type (RCE), their interaction (X) and residual variation among tree holes within treatments (TH).

Mean crowding

The vernal population consisted of *A. geniculatus* that hatched after the freeze and of *A. geniculatus* and *A. plumbeus* that had overwintered as larvae. Of the mosquito fauna in forty-two holes at Silwood Park and at Blacknest censused between 22 and 28 March 1986, *A. geniculatus* exhibited the highest mean crowding and had a higher mean interspecific crowding on *A. plumbeus* than *A. plumbeus* had on itself (Fig. 1A).

The aestival population of mosquitoes was evaluated from the contents of thirty-seven unmanipulated holes at High Standing Hill censused 21–27 July 1986 (Fig. 1B). In contrast to the vernal population, the community was dominated by *C. torrentium* with a mean intraspecific crowding of 745/litre. In the summer, *A. plumbeus* were more abundant than in the

spring and more abundant than contemporary *Aedes*. Only *Culex* encountered more of its own species than all other species combined and mean intraspecific crowding of *Culex* was 7 times greater than its mean interspecific crowding on either *Aedes* or *Anopheles*. In sum, *Culex* encountered mainly other *Culex*; *Anopheles* encountered about as many *Culex* as other *Anopheles*; *Aedes* encountered fewer *Anopheles* but more *Culex* than other *Aedes*.

Fitness and tree-hole type

Spring–summer (Experiment 1). When *A. geniculatus* developed in tree holes at a density of 337/litre, tree-hole type had no significant effect on pupation success (Fig. 3) ($F_{2,9} = 3.68$; $P > 0.05$). Two-way ANOVA showed that mean pupal weight (Fig. 4) of females was

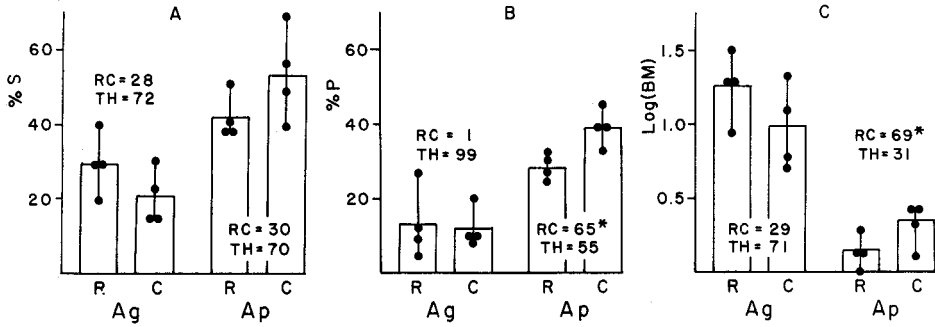


Fig. 5. (A) Survivorship (%S, arcsine transformed), (B) pupation success (%P, arcsine transformed), and (C) biomass yield (log BM) of *A. geniculatus* and *A. plumbeus* in rot and cryptic buttress holes during the summer and autumn 1986 at Silwood Park. The tables within each graph provide the percentage reduction in total sum of squares from the ANOVA attributable to tree-hole type (RC) and to residual variation among tree holes within tree-hole types (TH).

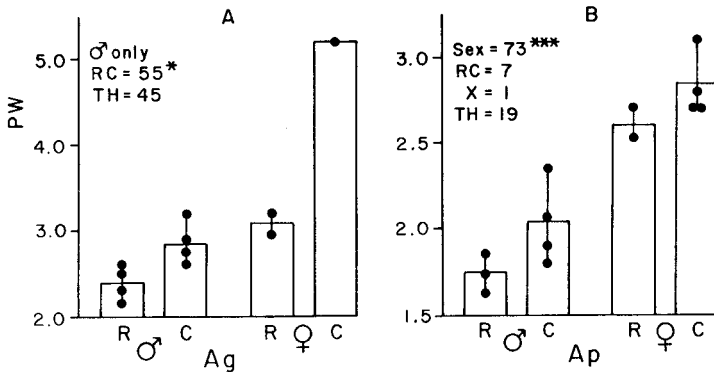


Fig. 6. Mean pupal weight (PW) of *A. geniculatus* and *A. plumbeus* in rot and cryptic buttress holes during the summer and autumn 1986 at Silwood Park. The tables within each graph provide the percentage reduction in total sum of squares from the ANOVA attributable to male–female differences (Sex), tree-hole type (RC), their interaction (X) and residual variation among tree holes within treatments (TH).

heavier than males ($F_{1,18} = 107.48$; $P < 0.001$), that pupal weight also differed significantly among tree-hole types ($F_{2,18} = 3.57$; $P < 0.05$), but that there was no sex:tree-hole type interaction ($F_{2,18} = 2.04$; $P > 0.05$). Applied to mean pupal weights, Duncan's Multiple Range Test failed to reveal significant differences between any two means from different tree-hole types. Mean development time (Fig. 4) for females was longer than for males ($F_{1,18} = 10.82$; $P < 0.01$) but did not differ among tree-hole types ($F_{1,18} = 1.06$; $P > 0.05$) or reflect any

significant interaction between sex and tree-hole type ($F_{2,18} = 0.40$; $P > 0.05$).

Summer–autumn (Experiment 2). Aedes. Among *A. geniculatus*, neither survivorship ($F_{1,6} = 2.36$; $P > 0.05$), pupation success ($F_{1,6} = 0.08$; $P > 0.05$) nor biomass yield ($F_{1,6} = 1.96$; $P > 0.05$) differed between rot and cryptic buttress holes (Fig. 5A–C). Male pupal weight (Fig. 6A) was greater in cryptic buttress holes than in rot holes ($F_{1,6} = 7.33$; $P < 0.05$). There were insufficient female pupae to test for the effect of tree-hole type on female pupal weight.

Anopheles plumbeus were reared in the same holes as *Aedes geniculatus* with an initial density of 13.3/litre; redistribution of recruiting first and second instars during each of six biweekly censuses resulted in a maximum density of about 15.3/litre in mid-September. Pupation success ($F_{1,6} = 10.89$; $P < 0.05$) and biomass yield ($F_{1,6} = 6.55$; $P < 0.05$) but not survivorship ($F_{1,6} = 2.57$; $P > 0.05$) was affected by tree-hole type with greater pupation success occurring in cryptic buttress than rot holes (Fig. 5A–C). Mean pupal weight (Fig. 6B) was heavier among females than males ($F_{1,9} = 64.54$; $P < 0.001$) and heavier in cryptic buttress than in rot holes ($F_{1,9} = 5.53$; $P < 0.05$) but there was no significant interaction between sex and tree-hole type ($F_{1,9} = 0.12$; $P > 0.05$).

Discussion

Among the overwintering larvae, survivorship of *Anopheles plumbeus* (Fig. 2D) was greater than survivorship of *Aedes geniculatus* (Fig. 2A) at each locality. Thus, larvae of *A. plumbeus* that overwinter exclusively as larvae exhibit greater net freezing resistance than larvae of *A. geniculatus* that may overwinter either as larvae or embryos.

Aedes geniculatus (Fig. 2A) showed the greatest survivorship in apparently sheltered rather than exposed habitats, i.e. along a large lake at Blacknest (BN) or on a south-facing slope at High Standing Hill (HS) rather than the unprotected areas at Silwood Park (SP) or on an exposed ridge at High Standing Hill (HR). At Silwood Park (Fig. 2A, SP) where *A. geniculatus* suffered its greatest mortality, survivorship increased with apparent tree-hole exposure (Fig. 2C), being higher in exposed than in cryptic buttress or rot holes, respectively.

Anopheles plumbeus (Fig. 2D) showed greater survivorship at both High Standing Hill localities (HR, HS) and along Virginia Water at Blacknest (BN) than at Silwood Park (SP). At Silwood Park (Fig. 2F), *A. plumbeus* survived better in rot than cryptic buttress holes. Thus, freeze-related mortality in both species was due to species-specific factors within each type of tree hole. *A. plumbeus* does, however, appear to be found in holes that are safer during freezing periods than holes occupied by *A. geniculatus*: *A. plumbeus* (Fig. 2E) survive better in holes they occupy exclusively (–AG) than those they

share with *A. geniculatus* (+AG) but *A. geniculatus* (Fig. 2B) survive better in holes they share with *A. plumbeus* (+AP) than those they occupy alone (–AP).

The effect of tree-hole type on annual fitness of *A. geniculatus* may be inferred from fitness indices during the winter, spring and summer–autumn. Freeze-related larval mortality increased in exposed buttress, cryptic buttress, and rot holes from 7% to 55% to 76%, respectively. However, as a fraction of annual recruitment from Table 1, freeze-related larval mortality represented a less dramatic 0.8%, 4.3% and 4.3% of the annual population in those respective holes.

After winter freezing and during subsequent development and metamorphosis, *A. geniculatus* achieved similar pupation success (Fig. 3), mean pupal weight and development times (Fig. 4) regardless of tree-hole type. During the subsequent summer and autumn, *A. geniculatus* did not achieve significantly different survivorship, pupation success, or yield in rot than cryptic buttress holes (Fig. 5A–C) although male pupal weight (Fig. 6A) was heavier in cryptic buttress than rot holes. Summer–autumn populations of *A. geniculatus* were lower than earlier in the year (Table 1; Kitching, 1971; Yates, 1979). Differences in annual fitness between rot and cryptic buttress holes due to differences in male pupal weight in the summer–autumn are therefore likely to be small but are opposite in effect and may offset some of the freeze-related mortality between rot and cryptic buttress holes. Thus, *A. geniculatus* probably realizes little or no difference in annual fitness among tree-hole types at Silwood Park.

Among *A. plumbeus*, survivorship (Fig. 5A) did not differ between rot and cryptic buttress holes. Pupation success (Fig. 5B), pupal weight (Fig. 6B) and yield (Fig. 5C) were higher in cryptic buttress than in rot holes late in the summer and autumn but overwintering *A. plumbeus* survived freezing better in rot than cryptic buttress holes (Fig. 2F) so that there is a seasonal reversal in tree-hole type in which *A. plumbeus* achieve higher fitness. *A. plumbeus* do not recruit heavily into exposed buttress holes and we have no means to gauge their relative fitness in exposed buttress holes.

The above discussion indicates that both species realize similar annual fitness among tree-hole types. This conclusion has two im-

portant implications. First, censuses or experiments centred about a single date or season may yield misleading information about fitness variation among tree-hole types. Second, both species should recruit into a broad range of tree-hole types. While *A.geniculatus* does recruit into the full range of tree holes, *A.plumbeus* recruits into (Table 1) and prevails in (Bradshaw & Holzapfel, 1986) predominantly rot holes. In *A.plumbeus*, greater recruitment into rot than cryptic buttress holes is not likely due to competition from *A.geniculatus* since greater fitness of *A.plumbeus* in cryptic buttress than rot holes (Figs 4 and 5) occurred in the presence and uniform densities of *A.geniculatus*. In southeastern North America, *Anopheles barberi* exhibits similar habitat segregation from *Aedes triseriatus* in tree holes (Bradshaw & Holzapfel, 1983, 1988). Habitat utilization, like specialized surface feeding, is probably a genus, not species specific trait and may reflect their phylogenetic history or the specific physiological and chemical attributes of tree-hole types rather than competition within those holes.

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