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Deadlines and delays as factors in aphid sex allocation

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Aphididae, sex ratios, sequential hermaphroditism

Abstract. In this paper we use the concept of the evolutionary individual as the basis for an attempt to characterise sex-allocation patterns in aphids. We then examine the various selection pressures involved in the evolution of aphids' sex ratios, and propose a novel explanation for biased sex allocation in host-alternating aphidines, in which inbreeding is impossible. Their production of gynoparae (females that migrate from secondary to primary hosts to produce the sexual females) before males is the clonal equivalent of sex reversal – sequential hermaphroditism. Selection on the timing of the reversal, and thus the overall sex-allocation ratio, should depend largely on the rate of decline of the populations on the secondary hosts in autumn and on how long it takes oviparae (sexual females) to reach maturity. The longer the nymphal period of the oviparae, the sooner investment in gynoparae becomes futile, since both gynoparae and oviparae must mature and oviparae must oviposit before leaf-fall. It is the combination of a deadline – leaf-fall – and a delay – the two generations that must be completed between allocation to gynoparae and oviposition – that determines the selection on the allocation ratios of host-alternating aphidines. Data on *Rhopalosiphum padi* in Scotland and Sweden show a strong female bias, as the model predicts.

INTRODUCTION

Current theories of sex allocation suggest two main reasons why organisms might deviate from the equality of investment in sons and daughters predicted by Fisher (1930). First, if sib mating is common, local mate competition (LMC) among related males may favour a female-biased allocation ratio (Hamilton, 1967); in other words, if most females are likely to mate with their brothers, a parent can maximize its number of grand-daughters by producing many daughters and few sons. Second, if the benefits of group living depend on the sex of the group's members, selection may favour a bias towards the sex contributing most to its relatives' fitness ("local resource enhancement"; Schwarz 1988).

Yamaguchi (1985) was the first to consider biased sex-allocation in aphids. She argues as follows. In Pemphiginae (and Hormaphidinae) the sexual morphs do not feed, and mate in the first or second instar. The two sexes thus have little time to disperse on the host before mating. This fact, and the likelihood that in some years sexuparae (the mothers of the sexuals) will be scarce or widely scattered over the host, means that many oviparae will be inseminated by their genetically identical brothers. This inbreeding or, more precisely, clonal self-fertilisation (Ward, 1991), results in the evolution of a female-biased investment ratio. Yamaguchi also extended Hamilton's (1967) model to show that the absolute investment in sons should be independent of the size (and thus reproductive output) of the sexupara: as predicted, large sexuparae produce more oviparae than males, while the sex ratio among the offspring of smaller sexuparae is less biased.

Local mate competition, therefore, may be a major factor in the evolution of biased sex allocation in pemphigines. Not all aphids, however, have life cycles likely to promote selfing; indeed, as we argue below, that of some aphidines precludes it. Yet biased sex-allocation ratios are common. In this paper we begin by defining the sex-allocation ratio in aphids, before discussing the relation between the life cycle, clonal selfing and local mate competition. We then go on to examine the sex-allocation pattern in host-alternating aphidines, in which selfing is impossible; and we discuss the implications of a model sex ratios in aphidines that host-alternate and those that do not.

What is a sex-allocation ratio in aphids?

Clearly, the fact that all parthenogenetic aphids happen to be female has no bearing on the question of sex-ratio evolution. But what is the sex-allocation ratio of a cyclical parthenogen? Elsewhere (Ward & Wellings, 1994) we have argued as follows.

Sex allocation is measured as the investment of limiting resources in sperm and ova and the machinery needed to produce them. In a hermaphrodite this machinery is the gonads etc.; in a dioecious organism the machinery is the sons or daughters, and the investment is measured up to the time they reach independence but does not include resources that the offspring acquire for themselves. In both of these cases the three main features of the calculation of the investment ratio are:

(1) A resource has been invested in, say, sperm once it can no longer contribute to the production of future ova.

(2) Investment is calculated at the time the resources are committed – in hermaphrodites the sex-allocation ratio is the ratio of the investments in sperm and ova, and the machinery required to produce them; in dioecious species it is the ratio of the investments in sons and daughters.

(3) Allocation is expressed as a fraction of the resources available at the time that they are committed to one or other type of gamete.

Turning now to sex-allocation by clones of aphids, we must distinguish between two forms of life cycle. In the Pemphiginae, the sequence of parthenogenetic generations culminates in the production of "sexuparae" which produce both males and sexual females. Resources are not irreversibly committed to future sperm or ova until the sexuparae begin investing in their offspring. The overall allocation ratio can thus be calculated from the biomass ratio of male to sexual female. A very different life cycle is seen in host-alternating aphidines. Here, sexual females are produced by "gynoparae", while males are produced by females of the previous generation – "virginoparae". Resources are thus already committed to future ova when they are invested in gynoparae; the appropriate ratio is that of males to gynoparae.

APHIDS' LIFE CYCLES, SELFING AND LOCAL MATE COMPETITION

How prevalent is selfing in aphids? All oviparae – the sexual females – are apterous, so for them to mate with a male from their own clone (1) they must be born on the same host as the males from their own clone; (2) these males must not emigrate before mating; and (3) there must be few other male-producing clones on the same host (or the same branch, if the host is a tree). In the pemphigine life cycle, apterous dwarf males and oviparae are produced by the same migratory sexupara; as Yamaguchi argues, this should result in high levels of selfing and LMC.

At the other extreme, in the host-alternating aphidines the males and oviparae are produced on different species of host: males are born on the summer (or "secondary") host, while oviparae are produced on the winter ("primary") host by migratory "gynoparae"; selfing is possible only if a male and a gynopara from the same clone chance to settle in the same individual host.

The level of selfing is likely to vary considerably among the many species of aphids with undwarfed sexuals but without host alternation (Ward, 1991): in aphids that feed on herbacious hosts – especially annuals – and whose males are apterous (e.g. some races of *Acyrthosiphon pisum*), an individual host may normally carry members of only a single clone, so selfing should predominate; in non-host-alternating tree-dwelling aphids such as *Drepanosiphum platanoidis*, members of several clones are likely to be present on the same host, so outbreeding may be common. [Here, the inbreeding coefficient will depend also on the coefficient of relatedness among the clones on each tree: they are full sibs if they come from the same ovipara; half-sibs if they are descended from co-clonal oviparae but unrelated males, etc. (Ward & Wellings, 1994).]

This argument suggests that aphids should show the full range of inbreeding rates – from full selfing (and thus strong LMC) in some non-host-alternating species on herbs to obligate outbreeding (and no LMC) in host-alternating aphidines. In what follows we concentrate on those aphids whose sex-allocation ratio cannot be affected by LMC – the host-alternating aphidines.

SEX ALLOCATION IN HOST-ALTERNATING APHIDINES

Sex determination and the progeny sequence

An aphid's sex is determined by the number of X chromosomes it has: females are XX (or $X_1X_1X_2X_2$), while males are X0 (or $X_1X_2 00$). Experiments on *Myzus persicae* have shown that sex determination is influenced by juvenile hormone, although the timing and mechanism of the hormone's effect on the X chromosome are still unclear: if an aphid's JH titre is low she produces male embryos (Hales & Mittler, 1983, 1985, 1987).

Perhaps as a consequence of this hormonal control, aphidines do not simultaneously produce both male and female embryos. Instead, sons and daughters are produced in discrete batches. There is normally an initial phase of daughter-production, followed by the batch of sons and, under some conditions, a series of daughters that continues until death: e.g. *Acyrthosiphon pisum* (Lamb & Pointing, 1972, 1975; MacKay et al., 1983; MacKay, 1987), *Cryptomyzus* spp (Guldemond & Tigges, 1992), *Megoura viciae* (Lees, 1960), *Myzus persicae* (Matsuka & Mittler, 1979; Takada, 1982a, 1982b; Hales et al., 1989), *Rhopalosiphum padi* (Dixon & Glen, 1971; Simon et al., 1991) and *Sitobion avenae* (Newton & Dixon, 1987).

The sex-allocation strategy of an evolutionary individual

In choosing ways of formalizing the problem we have found it helpful to use Janzen's (1977) concept of the "evolutionary individual." This refers to all members of a single clone: the evolutionary individual is "born" as a fundatrix, "grows" by increasing the number of bodies of which it is composed, and "reproduces" sexually, producing a new evolutionary individual in each egg. It has not only genetic but also developmental integrity. Lees' (1960) "interval timer" prevents the switch from parthenogenesis ("growth") to sex ("reproduction") until the clone has reached a certain age, irrespective of the number of parthenogenetic generations it has passed through; the evolutionary individual thus has its own ontogeny, distinct from, and transcending those of its constituent bodies (Ward & Wellings, 1994).

What then is the sex-allocation strategy of an aphid clone? The alternatives are: dioecy, in which each individual functions only as a male or only as a female; simultaneous hermaphroditism, where an individual invests simultaneously in sperm and ova; and sequential hermaphroditism, in which each individual changes sex.

We are aware of only two cases of clonal dioecy in the Aphidoidea: the androcyclic clones of *Myzus persicae*, which produce males but no sexual females (Blackman, 1976) and are thus male evolutionary individuals; and european populations of *Gilletteella coweni* (Adelgidae), which throw sexual females but no males (Steffan, 1968). In general, aphid clones are hermaphrodites. The pemphigines, in which each sexupara produces both males and sexual females, are simultaneous hermaphrodites – a reclassification which does not affect the validity of Yamaguchi's predictions. Sex allocation by host-alternating aphidines, however, can best be described as sequential hermaphroditism: gynoparae are produced before males, so resources are committed first to future ova, then in future sperm: the clone undergoes sex reversal, from female to male. (Since different virginoparae may switch at different times the clone may also pass through a period as a simultaneous hermaphrodite.)

This analogy – between aphidine sex allocation and the sequential hermaphroditism of one-bodied individuals – suggests three questions (Charnov, 1982) that must be answered if we are to understand the evolution of aphidine sex allocation:

(1) Which sex should be first?
(2) When should the clone change sex?
(3) Should all clones change at the same time?

We turn now to the assumptions and predictions of a game-theoretic model for the evolution of clonal sex reversal (Ward & Wellings, in prep.).

Deadline and delay

The important details of the autumn stages of the life cycle are as follows.

The switch from parthenogenesis to investment in males and gynoparae is cued, in many species, by declining daylength and temperature (Dixon & Glen, 1971). Males or gynoparae are born on the summer host, develop to maturity and migrate to the primary host, where the gynoparae give rise to the oviparae. These must then reach maturity before they can mate and oviposit. There is thus a lag of one generation from cue to copulation if the clone produces males, and a two-generation lag between cue and oviposition if it produces gynoparae. In *Rhopalosiphum padi*, the details of the cue mean that there is just enough time for gynoparae and oviparae to mature before leaf fall – the deadline for oviposition (Ward et al., 1984). We assume that this deadline applies also to other aphidines.

This combination of the ecological deadline and the difference between the delays in reproduction through male and female function means that gynoparae should be produced before males: as the deadline nears, there comes a time after which gynoparae are valueless but males still have time to mature, migrate and mate; conversely, males born at the start of the sexual phase must wait until both the gynoparae and the oviparae have matured before they can mate, so are likely to suffer heavy mortality.

Given that gynoparae should precede males, when should the clone switch? This depends on the temporal change in the value (in numbers of overwintering eggs) of the two morphs, which depends in turn on their longevity and the breeding sex ratio. This latter depends on the distribution of switch times among clones and on how many of the aphids on the summer hosts survive to produce males. The model must thus include not only the lag terms and the deadline but also a description of the changes in density of the population on the summer hosts. For simplicity we assume that the population on the summer hosts declines exponentially once sex has been cued; that males, gynoparae and oviparae have different but constant instantaneous mortality rates; that gynoparae reproduce at a constant rate from maturity to death; and that the mating (and thus oviposition) rate is proportional to the product of the densities of males and oviparae.

We seek the evolutionarily stable distribution of switch times by first iterating to find the stable pure strategy (i.e., assuming that all clones switch at the same time), testing the stability of this strategy against invasion by any others and, if it is unstable, allowing the system to evolve (by simple competition among "clones" with different switch times) until a stable mixture is reached.

General predictions

The model yields a number of predictions about the way the sex-allocation ratio at the evolutionarily stable strategy (ESS) should depend on the various ecological parameters.

First, the ESS may be pure, in which case all clones switch at the same time, or a mixture of two switch times. With realistic values for the length of the delay – i.e. with the ovipara's development time greater than 10% of the time between the cueing of sex and the deadline for oviposition – the ESS is pure. If there is no lag, the model predicts that half of the clones should invest immediately in males and the rest only in gynoparae: the ESS reverts to Fisherian equality of investment.

Second, the ratio of the accumulated investments in males and gynoparae may be male-biased, equal or strongly female-biased. The parameters with the greatest influence on this ratio are the rate of decline of the population on the summer host – the mortality of the virginoparae – and the length of the delay. Generally, over the range of realistic values assumed for these parameters, the accumulated allocation ratio at ESS becomes more female-biased as each parameter is increased. The longer the oviparae take to reach maturity, the longer early-born males must wait for mates, so the later the stable switch time becomes – the greater the investment in gynoparae. And the more rapidly the source population on the secondary hosts declines the fewer virginoparae survive to produce sons; clones should switch earlier, but the effect of this change is outweighed by the decline in the number of virginoparae.

Measuring sex allocation

Before considering the results of field surveys and laboratory experiments we must note that there are some important methodological problems in the measurement of sex allocation in aphidines:

(1) Aphids live longer in the laboratory than in the field. This means that laboratory studies are likely to yield results biased towards the sex that is produced last – males. This may be one reason why laboratory studies have often yielded even or male-biased allocation ratios (e.g. Newton & Dixon, 1987; Hales et al., 1989; Guldemond & Tigges, 1992).

(2) The switch to the production of gynoparae and males is stimulated by a complex combination of changes in daylength and temperature. Different treatments yield different sex-allocation ratios, so it is difficult to derive a realistic measure of sex allocation from experiments in the laboratory.

(3) Males may be more vagile than gynoparae (Ward, Leather & Pickup, in prep.), so samples on the host plant may underestimate, and suction-trap catches overestimate, their relative abundance.

Data

As a preliminary test of the model we now use field estimates of the major parameters to predict the sex-allocation ratio in the bird cherry-oat aphid, *R. padi*. The developmental time of the oviparae relative to the time from induction of sex to leaf-fall must normally lie between 0.2 and 0.33; and we estimate the mortality of *R. padi* virginoparae as: $3.2 < m_vT < 4.0$, where m_v is the instantaneous mortality among virginoparae and T is the time between induction of sex and leaf-fall. Wherever the parameters lie within these limits, the model predicts a female-biased allocation ratio; its exact value, however, is very sensitive to the parameters – the predicted investment in males varies from 15% to 50%, depending on the chosen value of m_vT. Wiktelius (1987) presents sex ratios for *R. padi* in Sweden (1975–1979) estimated from suction-trap data; we have also calculated sex ratios using similar data from Scotland (1982–1987; Ward, Leather & Pickup in prep.). Males and gynoparae are of equal weight at birth (S.R. Leather, pers. comm.), so the ratio of investment should equal the sex-allocation ratio. The estimates vary greatly between years, but all but one (Scotland, 1984) are strongly female-biased: in Sweden males constitute 7.9% of the population of autumn migrants (range = 5.8–37.7); in Scotland 14.9% (range 4.8–82.4).

The errors in estimation of the parameters and the variability in the results means that although the model's quantitative predictions are not refuted we cannot argue that they are confirmed. This will require far more accurate measurement of the main parameters in the field. The data do, however, agree with the qualitative prediction: that the out-breeding *R. padi* should invest less in males than in gynoparae.

DISCUSSION AND CONCLUSIONS

The arguments above apply to aphidines that alternate between primary and secondary hosts. What of the 90% of aphidines that are autoecious – that colonise the same species of hosts throughout the year (Dixon, 1985)? In particular, how important are local mate competition and the deadline/delay effect postulated above?

The effect of the delay will probably vary considerably among autoecious aphidines. It may be important in species such as *Sitobion avenae*, which has gynoparae whose offspring are all oviparae (Newton & Dixon, 1987). In species in which the males and oviparae can be produced by the same mothers, such as the non-host alternating race of *Cryptomyzus galeopsidis* (Guldemond & Tigges, 1992), the time between the commitment of resources and the production of ova is no longer than the lag between investment and sperm production, so the sex-allocation ratio should not depend on the factors important in host alternators. As we argue above, the degree of selfing, and thus local mate competition, will also vary greatly among species, being perhaps very important in those that feed on herbaceous hosts and whose males are apterous but having little effect on species that feed on large long-lived hosts. The evolution of sex-allocation patterns in autoecious aphidines, therefore, is likely to depend on both local mate competition and the deadline/delay effect to a degree that varies considerably among species.

While it remains unclear to what extent the factors discussed above apply to autoecious species, they will almost certainly influence the selection on sex-allocation in host-alternating aphids. With apterous oviparae, all such aphids must produce either sexuparae or alate males and gynoparae. In species with sexuparae, selfing may result in local mate competition, at least in some years on some hosts, and thus cause the evolution of the female-biased allocation ratios observed in pemphigines (Yamaguchi, 1985). In species with gynoparae and alate males, there is a two-generation lag in the production of ova but only a one-generation delay in the production of sperm. This, together with the need to complete oviposition before leaf-fall, means that the stable sex-allocation pattern is the clonal equivalent of sex reversal, with an overall bias that depends on details of the population dynamics on the secondary hosts. Fisherian equality of investment (Newton & Dixon, 1987) is a mere coincidence, not a central prediction of the theory.

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