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REVIEW

Reproductive ecology of *Drosophila*

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Summary

1. Species of the genus *Drosophila* reproduce in a wide range of different resources, including fruits, sap, flowers, mushrooms and cacti. *Drosophila* species and their resources also exhibit considerable variability in geographic distribution.

2. Habitat and resource differences pose enormous challenges for *Drosophila* species. Host chemistry may include highly toxic compounds and breeding sites may be characterized by extreme abiotic conditions such as high and/or low temperature and humidity.

3. *Drosophila* reproductive biology, in terms of morphology, physiology, and behaviour, is as variable among *Drosophila* species as is their resource use. In some species, adults are ready to reproduce upon emergence, whereas one sex or the other in other species may require weeks to become sexually mature.

4. Already a robust system for transmission and population genetic studies, the sequencing of the genomes of 12 diverse *Drosophila* species now brings the power of genomics to investigators wishing to understand the functional aspects of *Drosophila* ecology

Key-words: ecology, reproduction, *Drosophila*, genomics

Introduction

The interaction between an organism and its environment involves a complex array of modalities that range from gene expression to population biology to community assembly. Functional ecology sits at the nexus of several disparate disciplines and serves as the unifying principle between evolutionary biology, genetics and genomics, and traditional ecological studies. Host plant preference is dependent upon the expression of a suite of genes determining timing of reproductive maturity and oviposition behaviour. Cues for these complex behaviours may be stimulated by plant chemistry or abiotic environmental factors. Phylogenetic and distributional constraints can have an impact on the specificity of host associations, population genetic structure and the rate of diversification within a lineage.

The *Drosophila* model offers a powerful framework of evolutionary and genetic studies to interpret the ecology of a given species. For example, *Drosophila* species differ widely in their levels of genetic diversity, nature and strength of selection at particular loci, and degree of population genetic structure. Combining this information with the considerable amount known about *Drosophila* ecology, we can meaningfully infer evolutionary processes underlying the observed patterns.

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There are over 2000 *Drosophila* species whose distributions range from narrowly restricted, single island endemics to panmictic, cosmopolitan taxa (Markow & O'Grady 2006). Species may feed and breed exclusively in resources such as flowers (Brncic 1983), mushrooms (Jaenike *et al*. 1983), fruits (Atkinson & Shorrocks 1977), leaves (Carson 1971), tree fluxes (Throckmorton 1975), cactus (Heed 1978), soil (Heed 1977) and even on land crabs (Carson 1974) or spider eggs (Hardy 1965). Certain *Drosophila* are specialists on a single host species, such as *D. sechellia* on the fruits of *Morinda citrifolia* (Jones 2005), or *D. pachea* on the rotting stems of the cactus *Lophocerus schottii* (Heed & Kircher 1965). Other species are generalists and will oviposit in several different hosts. The radiation of *Drosophila* onto such diverse resources has substantial implications for the reproductive biology of each species, which in turn influence the rate of diversification within a given lineage.

As with other insects, successful reproduction requires adult flies to be sexually mature, fertile, able to locate mates, and, finally, to oviposit at sites where fertilized eggs have the greatest likelihood of completing development to yield the next generation of adults. These reproductive processes may be influenced by factors such as resource chemistry, spatial and temporal abundance, and abiotic factors characteristic of the niche that the flies inhabit. Unlike the situation in many other insects, however, phylogenetic relationships of hundreds of *Drosophila* species are extremely well-defined and, coupled with the availability of whole genome sequences for

twelve diverse species (*Drosophila* 12 Genomes Consortium 2007), *Drosophila* provides a powerful system to study the interplay between ecology and evolution. Our understanding of the reproductive biology and ecology of the genus *Drosophila* as a whole provides unique opportunities to address general biological questions.

Reproductive biology of *Drosophila*

Before we can examine the role of ecological factors in the evolution of *Drosophila* reproductive strategies we first need to review some basic features of *Drosophila* reproduction. Adult flies emerge from pupa cases located either in the decaying plant material that had served as their larvae food or in the earth nearby. In the majority of species, newly emerged adults are not sexually mature (Markow 1996, 2002). In fact, sexual maturation may require up to several weeks, depending upon the species (Markow & O'Grady 2006). While males of some species mature more rapidly than females, in the majority of taxa examined, males mature more slowly than females. While much of the interspecific variability in reproductive biology reflects the evolutionary relationships of the species, many differences are not constrained by phylogenetic history (Pitnick, Markow & Spicer 1995). Sexual maturity is a function not only of the ability to execute the appropriate mating behaviours but of the possession of mature, functional gametes as well. For *Drosophila*, these represent two different, yet related, processes: gametogenesis and behavioural maturation.

GAMETOGENESIS: DEVELOPMENT OF SPERM AND OOCYTE

Although gonadal development in both sexes begins during larval and pupal stages (Bodenstein 1950), adults of various species emerge with their eggs and sperm at very different stages of maturation. While *Drosophila melanogaster* males emerge with mature sperm in their testes, this clearly is not the case in many other species (Pitnick, Markow & Spicer 1995; Pitnick 1996). Males of some species produce very long sperm, requiring a protracted testicular growth period due to accommodate the lengthened spermiogenesis process (Pitnick, Markow & Spicer 1995). Sperm elongation, for example, requires nearly 2 days in *D. melanogaster*, a species with sperm of 1·9 mm. This process takes over three days in *D. hydei*, a species that makes a very long sperm, 23 mm. Other species, such as *D. bifurca*, makes an even longer sperm, 55 mm, and although the elongation phase has not been measured, it is likely to be considerably longer than in *D. hydei.* Male sexual maturity in these three species is clearly associated with sperm length and the total amount of time required to produce and transfer mature sperm. The sperm maturation process lasts 2 days in *D. melanogaster*, 9 days in *D. hydei* and 3 weeks in *D. bifurca* (Pitnick, Markow & Spicer 1995). Thus the proximate explanations for species differences in male maturation reside in the length of time required for gametogenesis.

Gametogenesis in females also exhibits substantial interspecific variability. *Drosophila* oocytes mature in ovarian follicles containing 15 highly polyploid nurse cells that supply the developing oocyte with RNA, proteins, and other material. Oogenesis has been divided into discrete stages, from 1 to 14 (reviewed in King 1970), simplifying the measurement of ovarian maturity at the time of emergence. Stages 1–7 are previtellogenic and take place before the uptake and deposition of yolk in the developing oocyte. The differences between stages 1 and 7 are based primarily on the degree of polyploidization of the associated nurse cells. Vitellogenesis, or yolk deposition, begins in stage 8, with the synthesis of all of the external egg membranes being completed by stage 14. In rapidly maturing species like *D. melanogaster* oviposition can take place less than 2 days after the emergence of the female.

Oocyte stage at emergence is not dependent on nutrient levels supplied in the laboratory during larval development, suggesting that this trait is a fixed characteristic of a given species (Markow *et al*. 1999). In general females of most species emerge with oocytes at stage 7 or earlier. Rarely, a female of *D. funebris* or *D. melanogaster* has been observed to eclose with a stage 8 oocyte (Kambysellis 1968; King 1970). However, Kambysellis (1968) observed females of several species and reported that different taxa emerge with ovaries at very different previtellogenic stages. In some species, such as *D. fulvalinatea*, the most mature stage in emerging females is stage 1 or 2, while in other species the ovaries the ovaries contain predominantly stage 7 ovaries. In some Hawaiian *Drosophila* species, mature oocytes may not appear for almost 4 weeks, suggesting an attenuated ovarian development time in these taxa (Craddock & Kambysellis 1997). As with males, reproductive maturity in females appears to be a function of the timing of oogenesis.

BEHAVIOURAL MATURATION

Behavioural, as opposed to gonadal, maturity constitutes the ability to deliver and receive the appropriate courtship signals, which, depending upon the particular *Drosophila* species, may involve one or more of several sensory modalities: olfaction, auditory, tactile and visual (Markow & O'Grady 2005). Pheromones typically are some blend of hydrocarbons, profiles of which for both males and females commonly change, after emergence, to reflect both sexual dimorphism and maturity (reviewed in Ferveur 2005). Other aspects of sexual maturity also change with age: males approaching sexual maturity sometimes will follow a female but deliver incomplete courtship behaviours. Females also have courtship behaviours that include both rejection and acceptance signals either of which may employ olfactory, auditory, or visual modalities. Physiological control of behavioural maturity in *Drosophila* has not been extensively studied, but appears to be under the control of dopamine (Neckameyer 1998), juvenile hormone (Ringo & Pratt 1978; Postlethwait & Handler 1979; Bownes 1989; Wilson, DeMoor & Lei 2003), and ecdysteroids (Richard *et al*. 2005).

Control of female behavioural maturity relative to ovarian maturity is not well-understood. As indicated above, ovarian maturation rates vary tremendously among species. In most

species, ovarian maturity correlates positively with female insemination, indicating that both behavioural and gonadal maturity are, to some degree, correlated (Kambysellis & Craddock 1991). Manning (1962) noted, however, that while dietary restriction could retard ovarian development in *D. melanogaster*, it had no influence on the onset of female sexual receptivity. Females of some Hawaiian *Drosophila* species (Kambysellis & Craddock 1991) and of *D. melanogaster* and *D. simulans* (Markow 2000), however exhibit precocious insemination from forced matings in nature. Timing of sexual maturation influences phenomena such as operational sex ratio, which in turn influences sexual selection (Markow 2002). Other life-history characters, like ovariole number or testis size, also are thought to be associated with a species' reproductive potential (Wayne & MacKay 1998; Telonis-Scott, McIntyre & Wayne 2005). Although a negative relationship exists between egg size and number, larger-bodied females have been shown to allocate less of their resources to reproductive tissues compared to smaller-bodied females (Starmer *et al*. 2003). Understanding the ecological contexts in which these reproduction patterns are found therefore is essential for understanding their role in evolutionary processes.

Ecological control of *Drosophila* reproduction

What is the role of ecology in *Drosophila* reproduction? What are the long-term forces that have shaped the observed reproductive differences between species and how plastic are they in the face of present ecological variability? Long-term evolutionary factors are expected to reflect adaptation to particular ecological parameters, but present day abiotic and biotic factors vary greatly in space and time and possess the potential to influence reproduction. Reproduction can be constrained profoundly by multiple aspects of a species' ecology, including longitude and latitude, and resource characteristics such as community composition, chemistry and spatial and temporal abundance.

SPATIAL AND TEMPORAL DISTRIBUTIONS OF FLIES

Some *Drosophila* species are found on all continents while others are restricted to the tropics, deserts, specific islands, or very high latitudes (Markow & O'Grady 2005). Figure 1 summarizes the geographic distributions of the major species groups and genera in the family Drosophilidae. While some clades are quite widespread, and many groups contain one or more cosmopolitan species, there are some biogeographic patterns. The most prevalent is a division between Old World and New World taxa. Mapping distributions on a phylogeny is illustrative of broad scale patterns, but can be somewhat misleading in that some species within a widespread group may be narrowly distributed. Additional phylogenetic work with more extensive taxon sampling is needed to resolve these finer scale patterns.

Species distribution reflects the distribution of their particular resources as well as their particular combinations of ecophysiological tolerances. Different geographic regions and their abiotic features impact *Drosophila* reproduction directly (e.g. high temperatures leading to a decrease in fitness via water loss) or indirectly (e.g. low humidity conditions reducing the frequency and duration of breeding sites). Collection records from particular locations reveal that, like other insects (e.g. Tauber & Tauber 1981), *Drosophila* species vary in presence and abundance on a seasonal basis (e.g. see Patterson & Stone 1952; Cooper & Dobzhansky 1956; Mangan 1978; de Toni & Hofmann 1995; Breitmeyer & Markow 1998). Population cycles may reflect intolerance to seasonal abiotic factors like temperature and relative humidity or to biotic factors such as competition, predation, or resource phenology (Kimura 2004). In Scandinavia, for example, where *virilis* and *obscura* group species dominate, temperatures are below freezing for much of the year when neither flies or their resources grow (Lumme & Lakovaara 1983). In the Sonoran Desert, on the other hand, the cactophilic species of *Drosophila* face summer temperatures of nearly 50 °C (Gibbs, Perkins & Markow 2003), well-above what many of them are able to tolerate (Stratman & Markow 1998). Flies simply are not found in the desert when temperatures exceed the tolerance limits determined in the laboratory. Yet their resources are most abundant in the hottest summer months (Breitmeyer & Markow 1998), suggesting for these endemic specialists, thermal intolerance, rather than resource limitation, governs their population cycles.

DIAPAUSE

Seasonal fluctuations in abundance suggest that most *Drosophila* species undergo some period of dormancy. Dormancy in insects, referred to as diapause (Tauber & Tauber 1981), refers to an arrest at a 'resistant developmental stage' characterized by metabolic changes mediated by hormonal responses to environmental cues. While typically we associate diapause with winter, summer diapause also is known in insects but has been far less well-studied (Masaki 1980).

A given species may be univoltine (one generation per year) or multivoltine (multiple generations per year), depending upon the latitude at which the populations are found. Species arrest in the 'resistant developmental stage' which, for *Drosophila*, is most commonly the adult stage. The 'resistant developmental stage' involves resistance to factors like starvation and desiccation, in addition to temperature. Diapause in insects is often triggered by day length (Tauber & Tauber 1981), because it is a more reliable indicator of season than temperature, although temperature and nutrition influence diapause as well (Schmidt & Conde 2006). Although summer diapause has not been reported in *Drosophila*, it is likely that certain desert species undergo some sort of arrest in the summer (Breitmeyer & Markow 1998). Tropical species also exhibit seasonal population fluctuations (Pipkin 1965; Mangan 1978). Observations on natural populations of *D. melanogaster* (Schmidt & Conde 2006; Schmidt, Paaby & Heschel 2005a; Schmidt *et al*. 2005b), however, suggest that the duration of dormancy and the accompanying metabolic changes may be very different from what characterizes winter

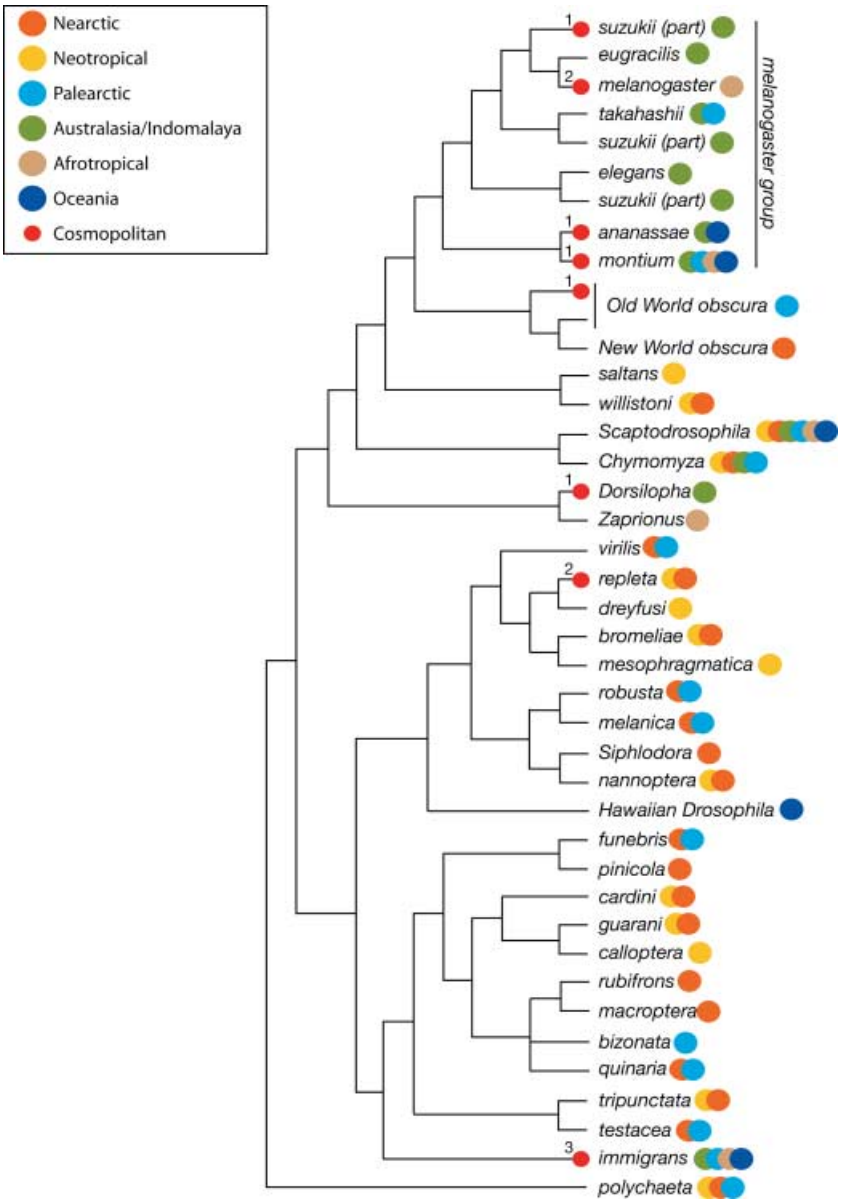

Fig. 1. Geographic distributions of *Drosophila* found in each of 40 species groups. Cosmopolitan species are human commensals, found on more than one continent.

diapause in temperate and arctic species. Diapause has been investigated most thoroughly in the temperate *virilis*, *obscura* and *quinaria* species groups (Lumme & Lakovaara 1983).

Drosophila can be an important model to help better understand diapause in insects. Tauber & Tauber (1981) point out that the evolution of diapause and its genetics are obscure, but suggest that diapause has a tropical origin. The latitudinal distributions of populations of related species of *Drosophila* thus offer an useful system for disentangling yet unresolved questions about the induction of and release from diapause and the nature of its full range of physiological correlates at different life-history stages. Seasonal changes in temperature and in daylight also influence other rhythm-based behaviours of *Drosophila*. Circadian rhythms, for example, can influence reproductive processes such as vitellogenesis onset (Handler & Postlethwait 1977) and sexual activity (Hardeland 1972) and are regulated by an internal oscillator system (Hardin 2005) consisting of specific genes that exhibit significant interspecific sequence variation (Kliman & Hey 1993; Gleason & Powell 1997). Barometric pressure (Ankney 1984) and temperature (Glaser & Stanewsky 2005) both interact with the circadian clock to influence reproductive cycles.

Population cycles and number of generations per year carry important implications for population and conservation genetics. While tropical species, such as *D. ananassae* or *D. malerkotliana*, and cosmopolitan species like *D. melanogaster* or *D. simulans*, may have 10 or more generations per year, temperate species such as *D. montana* or *D. subobscura* may have only one. The number of generations a given species produces per year is a critical component in many routine population genetic tests (Kimura 1981; Hudson, Kreitman & Aguade 1987; Hudson 1990) and for predictions of how species may respond to global change (Crozier & Dwyer 2006).

RESOURCE AVAILABILITY

Drosophila are saprophytic insects and, for the most part, utilize decaying plant material of all sorts. Although *Drosophila* are primarily associated with plants, they are not phytophagous insects in the strict sense. They feed upon the microbial community (bacteria, moulds, and yeasts) responsible for decomposition as well as upon the decomposed material itself. In some cases the microbes, through decomposition, make certain host components available to the *Drosophila* while they also may detoxify plant compounds that are otherwise harmful to *Drosophila* (Starmer 1981).

Although adults of most species will feed on a multiplicity of food sources, oviposition and larval development typically are more restricted (Carson 1971). For example, adults of a wide range of species can be attracted to simple banana baits fermented with *Saccharomyces cerevisiae*, but natural feeding and breeding sites are not necessarily similar to these baits. Figure 2 presents an overview of *Drosophila* breeding sites. The majority of *Drosophila* species belong to one of two large subgenera, *Sophophora* and *Drosophila* (Markow & O'Grady 2006). Species in the subgenus *Sophophora* tend to breed in many rotting fruits, although some utilize other substrates as well. Members of the subgenus *Drosophila*, on the other hand, occupy a far wider range of substrate types and display a greater diversity of resource specializations (Shorrocks 1982). Within any given species group, there may be species that are generalists and oviposit in a wide array of substrates,

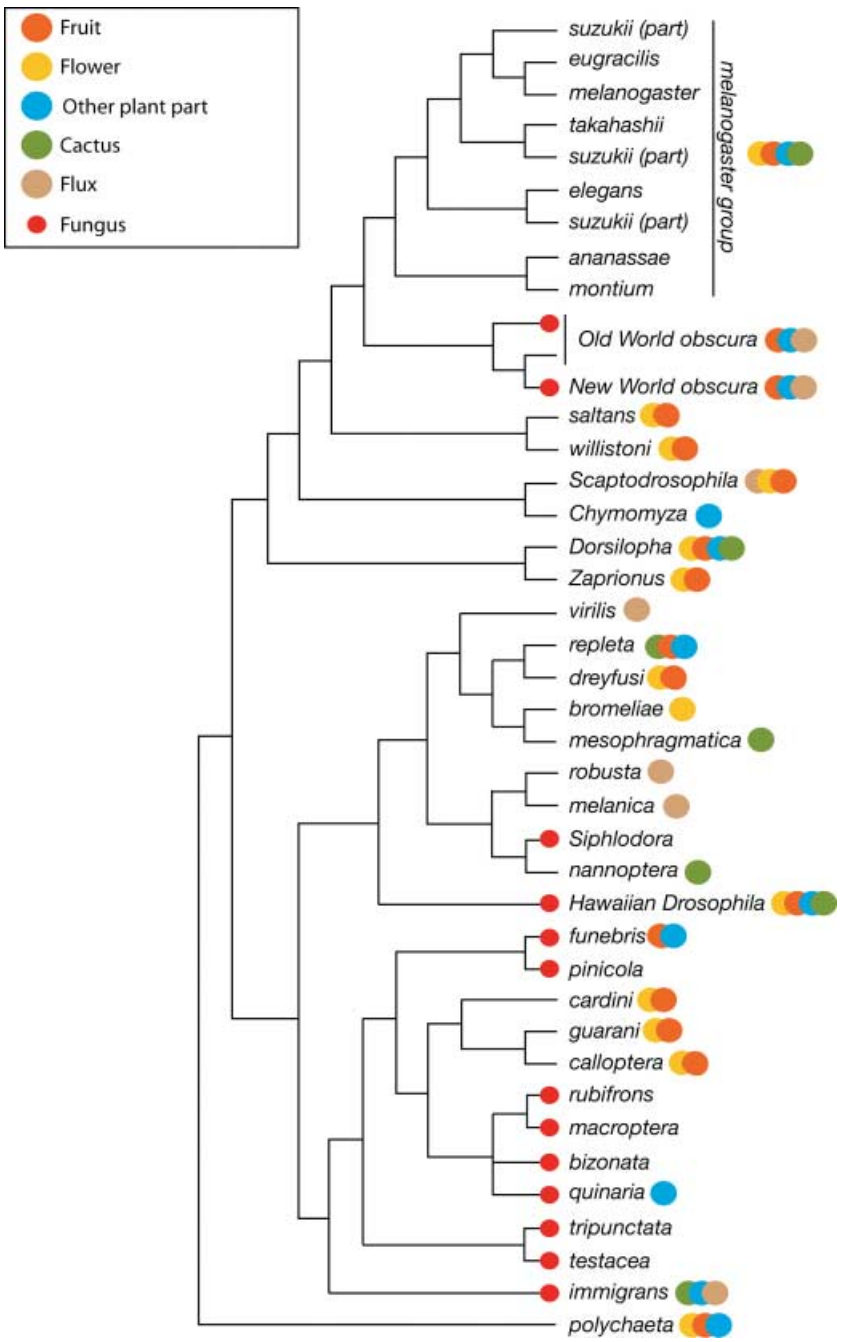

Fig. 2. Types of resources utilized by *Drosophila* in 40 species groups.

commensal taxa, including *D. melanogaster*, *D. simulans*, *D. funebris*, *D. virilis*, *D. hydei*, *D. pseudoobscura*, *D. immigrans* (Patterson & Stone 1952; Carson 1972; Powell 1997), associated with human activity, and species that have specialized on one particular plant or fruit (da Cunha, Shihata & de Oliveira 1957; Heed & Kircher 1965; R'Kha, Capy & David 1991; Spicer & Jaenike 1996).

Development times are also correlated with the primary host resource. Species ovipositing and developing in ephemeral hosts, such as fungi and flowers, tend to develop much more rapidly than those that are reliant upon longer lasting resources like necrotic cacti and rotting trees (Table 1). Cosmopolitan species (Fig. 1) tend to also be rapid developers who can utilize a wide range of host types (Table 1). Species of Hawaiian *Drosophila*, which oviposit in native Hawaiian tree species, relatively resource-poor substrates, tend to have much longer development times (Markow & O'Grady 2006).

SPECIALIST AND GENERALIST LIFESTYLES

Those species that have been sufficiently studied appear to support the standard rule: monophagy occurs when food source is highly predictable, polyphagy is found when less predictable. This relationship is exemplified by *D. quinaria and D. falleni*, respectively (Jaenike 1990). *Drosophila quinaria* breeds in skunk cabbage (*Symplocarpus foetidus*), which is highly abundant throughout the spring, summer, and fall at the same locations. Mushrooms, however, are less predictable and mycophagous taxa, such as *D. falleni*, utilize a wide variety of mushroom species, rather than relying upon a single host taxon. Some *Drosophila* species, such as *D. phalerata* and *D. subobscura* (Shorrocks 1982), partition a given host. These two taxa breed in different parts of the stinkhorn mushroom, *Phallus impudicus*. *Drosophila mettleri* and another cactophilic species, *D. nigrospiracula* both utilize the saguaro *Carnegiea gigantea*, and cardón *Pachycereus pringlei*, but the former oviposits in the soil soaked by the necrotic juice of these cacti, while the latter breeds in the necrotic tissue itself (Heed 1982). *Drosophila silvarentis* and *D. heedi*, two Hawaiian species, similarly divide their host substrate, with the former ovipositing in the sap flux of the *Myoporum sandwichenses* and the latter in the soil soaked by the dripping sap (Kaneshiro *et al.* 1973).

MICROBIAL ECOLOGY OF *DROSOPHILA* HOST PLANTS

Prior to Sang's (1956) and Royes' & Robertson's (1965) studies of various macromolecules in laboratory *Drosophila* diets, other researchers were investigating the microbes associated with *Drosophila* feeding and breeding sites in nature (Wagner 1944; Hedrick & Burke 1950, 1951; Shihata, Mrak & Phaff 1955; Carson *et al.* 1956; Phaff *et al*. 1956). Among the most detailed studies of yeast communities associated with *Drosophila* resources (Begon 1982) were those of Heed and his associates on the cactophilic species (Heed *et al*. 1976; Starmer *et al*. 1982) and of Begon & Shorrocks (1978) on the woodland species. Physiological attributes of the yeasts represent an integral feature of the habitat for the *Drosophila*. Different species of yeasts decompose different host constituents. Host differences in microbial composition thus reflect host chemistry. Similar yeast communities are observed in similar types of plant material, and these communities cluster with the evolutionary radiations of related *Drosophila* species

Table 1. Development time and substrate type by taxonomic group. Development times measured in the laboratory at 24 °C (Taken from Markow & O'Grady 2006)

Group	*N*	Mean time egg to adult	Substrate type	Notes
Immigrans	4	12	Fungi, fruits, flux, flower	Includes several cosmopolitan, generalist species
Quinaria	4	13·9	Fungi	
Testacea	1	14·5	Fungi	
Tripunctata	4	14·8	Fungi	
Zaprionus	3	14·8	Flower, fruit	
Bromeliae	1	15	Flower	
Cardini	9	15·1	Flower, fruit	
Willistoni	8	15·2	Flower, fruit	
Melanogaster	48	15·7	Fruit, flower, other plant part, cactus	Includes several cosmopolitan, generalist species
Obscura	8	16·1	Fruit, flux, other plant parts	
Calloptera	1	16·5	Flower, fruit	
Guarani	2	16·8	Flower, fruit	
Nannoptera	2	16·8	Cactus	
Repleta	41	16·9	Cactus	
Saltans	7	18·2	Flower, fruit	
Robusta	3	18·3	Flux	
Scaptodrosophila	5	18·7	Flux, flower, fruit*	Only flux breeders sampled
Mesophragmatica	2	19·3	Cactus	
Virilis	11	21·6	Flux	
Hawaiians	7	26·1	Other plant parts	

as well (Starmer 1981). Microbe-resource combinations thus determine which *Drosophila* species are present.

The role of bacteria in *Drosophila* ecology has received less attention than that of yeasts. The few existing surveys reveal a diversity of bacteria in *Drosophila* breeding sites and show that the bacterial community differs with the particular type of resource surveyed (Young, Vacek & Heed 1981; Oakeshott, Vacek & Anderson 1989; Foster & Fogleman 1993). Bacteria appear to be more important in the decomposition of plant material, while yeasts, especially the fermenting yeasts, are more important in fruits and cacti (Atkinson & Shorrocks 1977). Furthermore, particular bacteria have been demonstrated to increase fitness in *D. melanogaster* (Bakula 1969; Brummel *et al*. 2004).

Male and female *Drosophila* transfer both bacteria and yeasts to one another during mating (Starmer, Pereis & Fontedevila 1988). *Drosophila* species also deposit microbes at breeding sites through their faecal deposits and on the surfaces of oviposited eggs (Bakula 1969; Gilbert 1980). By inoculating new breeding sites with microbial flora, adults are, in effect, enhancing the resources available to their larvae when they later hatch from oviposited eggs.

HOST PLANT CHEMISTRY

Resource nutritional quality can be considered at two levels: (i) macromolecular, such as carbohydrates, proteins, lipids, nucleic acids and vitamins, and (ii) elemental, such as carbon (C), nitrogen (N), and phosphorus (P). To a certain degree, the two levels are related, as macromolecule classes differ in their relative amounts of C, N and P. *Drosophila melanogaster* require the same ten essential amino acids as other eukaryotes (Rudkin & Schultz 1947) and thus all *Drosophila* species are assumed to be similar in this regard. Both the microbes and the material they decompose provide sources of nutrition, but their relative contributions to growth and reproduction are yet to be determined for most species. In experiments on axenic controlled media, *Drosophila* species were found to differ significantly in their requirements for all of the major macromolecules (protein, carbohydrate, lipid), with marked effects on fitness traits such as development time and body size (Royes & Robertson 1965) reproductive output and aging (Tu & Tatar 2003). Some species differences still were observed even when yeast was added to the medium (Droney 1998). Because dietary-induced changes in body size are associated with differences in female ovariole number and hence egg production, larval nutrition clearly critical to subsequent adult reproductive success (Robertson 1957; 1959).

Adequate larval nutrition is insufficient for subsequent adult reproduction. Adult flies must feed to attain reproductive maturity. Emerging adult *D. melanogaster* females in fact require a complex diet to under vitellogenesis (Bownes, Scott & Shirras 1988): sugar alone is insufficient (Bownes & Blair 1986). The nature of the nutritional requirements for maturity clearly is species-specific. For example, ovarian maturation required only a third as many days in the mycophagous *D. phalerata* provided with mushrooms compared to conspecifics receiving only ordinary laboratory culture medium (Charlesworth & Shorrocks 1980). Mechanisms underlying dietary restriction and delayed oogenesis are not completely clear. Drummond-Barbosa & Spradling (2001) demonstrated, however, that while protein restriction does not alter the number of ovarian stem cells, it modifies, via the insulin signalling pathway, the rate at which progenitor cells proliferate or undergo apoptosis just before vitellogenesis. Genes potentially involved in sensing the nutritional deficiencies have been detected using micro-array and RT PCR (Terashima & Bownes 2005, 2004). Interestingly, females reared under nutritionally poor conditions make larger eggs, although eggs are fewer in number (Prasad *et al*. 2003). As functional genomics identifies biochemical pathways and interacting gene networks in model species, these can be applied to natural populations of generalist and specialist *Drosophila* species in order to better understand the interaction between oviposition choice, host quality and larval gene expression.

Wild-caught adult males and females of several species have been found to differ in the microbes isolated from their crops (Robertson *et al*. 1968), and in their stable isotope ratios of C and N (Markow, Anwar & Pfeiler 2000). The sexes therefore must frequent different feeding sites and have different nutritional requirements, likely related to their reproduction and or dispersal. Of all *Drosophila* species examined thus far, females have significantly higher levels of body phosphorus (Markow *et al*. 1999, Jaenike & Markow 2003). Females require excess phosphorus for the extensive gene amplification in nurse cells and the provisioning of oocytes with an abundance of transcripts (King 1970; Markow *et al.* 1999). In many *Drosophila* species, females supplement their diets through contributions of proteins (Markow & Ankney 1984; Pitnick, Markow & Spicer 1999) and phosphorus (Markow, Anwar & Pfeiler 2000) derived from male seminal fluid.

Larval growth rates have been examined in five *Drosophila* species for which the relative levels of CNP in their natural resources are known (Watts *et al*. 2005; Elser *et al*. 2006). Species whose natural hosts are low in levels of N and P exhibit a threefold increase in the time larvae require to pupate, compared to *D. melanogaster* and the mycophagous *D. falleni*, whose resources are N and P rich. A wider range of species using hosts of contrasting qualities will need to be examined before firm generalizations can be made.

SPATIAL AND TEMPORAL ASPECTS OF HOST AVAILABILITY

Drosophila resources also are highly variable in their spatial and temporal abundance and distributions and patch size may vary widely between species and season. Food sources may occur as large patches, such as at an orchard, a winery or a produce processing facility, where populations of *D. melanogaster* and *D. simulans* typically attain huge numbers (Penrose & Womeldorf 1962; Marks *et al*. 1980). At the other end of the spectrum are flower-breeding species, which have adapted to a more seasonal resource and that may accommodate only one female, on average, per flower (Brncick 1983). Even among cactophilic *Drosophila*, specific

hosts differ from occurring in abundant but small patches to scarce but large patches (Breitmeyer & Markow 1998). Larger patches of mushrooms support a greater number of flies (Worthen 1989), but desiccation tolerance, which can influence reproductive traits, differs among mycophagous *Drosophila* species (Worthen & Haney 2002).

Resource attributes have been linked to *Drosophila* reproductive traits in a range of species. For example, flower-breeding species have fewer ovarioles and correspondingly lower reproductive rates. Furthermore, females of flower-breeding species often are ovoviviparous or viviparous (Pipkin, Rodriguez & Leon 1966; Kambysellis & Heed 1971), depositing mature embryos or larvae, which then more rapidly complete the larval stages. As individual flowers each last only a short time, traits that expedite development would be advantageous. Interestingly, eggs of flower-breeders often lack the typical chorionic filaments, which are respiratory in nature. The lack of filaments may reflect the habit of larvaposition or exist because the eggs are laid on the resource surface where respiration is not a problem. Only a few larvae or eggs are laid on a single flower (Pipkin 1966).

HOST AND MATE LOCATION

How do flies locate their feeding and breeding sites? Microbial decomposition of host material creates volatiles that also serve to attract flies to the appropriate feeding and breeding sites (Reed 1938; Fuyama 1976; Farine *et al*. 1996; Fogleman 1982; Hoffmann & Parsons 1984; Newby & Etges 1998; Stensmyr, Dekker & Hansson 2003). While flies clearly are attracted to their breeding sites by the volatile compounds produced by fermenting substrates, in some species, mating does not take place on the fermenting substrate itself (Spieth 1978; Markow 1988; Tompkins, McRobert & Kaneshiro 1994; Droney & Hock 1998). Once at the resource, other mechanisms are likely to underlie the location where specific behaviours, such as mating and oviposition, take place. About a dozen *Drosophila* species have been found to produce aggregation pheromones (Shorey, Bartell & Browne 1969; Bartelt, Schaner & Jackson 1988, 1989; Schaner & Jackson 1992; Moats *et al*. 1987; Schaner, Graham & Jackson 1989b; Schaner, Tanico-Hogan & Jackson 1989c; Schaner *et al*. 1989a,c). In many of these species, the pheromone is found in the male ejaculatory bulb and transferred to the female, who releases it to the substrate or the immediate environment. In those cases in which it was examined, the aggregation pheromone acted synergistically with laboratory fly food to attract flies of both sexes. Natural resources, however, were not examined, but would be expected to produce similar synergistic interactions. Advantages to aggregation of mated females can accrue from oviposition in that a critical number of larvae are needed to 'work' the food as well as reduce the risk of parasitism and predation.

OVIPOSITION

Several aspects of oviposition are critical to a female's reproductive success. Most essential is oviposition site selection, which exhibits variation not only among but also within species. Females use a wide range of cues in choosing oviposition sites. Interspecific differences in oviposition site have been demonstrated to involve factors such as ambient light (Wogaman & Seiger 1983), host chemistry (Richmond & Gerking 1979; Lofdahl 1985; Amlou, Moreteau & David 1998; Fanara & Hasson 2001), host microbial composition (Hoffmann & Harshman 1985; Oakeshotte, Vacek & Anderson 1989), host texture (David 1970; Rockwell & Grossfield 1978; Fogleman, Hackbarth & Heed 1981; Chess & Ringo 1985), substrate temperature (Schnebel & Grossfield 1986a,b; Fogleman 1979) and presence or absence of larvae (Del Solar & Palomino 1966; Chess & Ringo 1985).

Jaenike (1985, 1990) proposed oviposition as consisting of two phases: settling and actual oviposition. Females may be attracted to a prospective oviposition site, settle on it and then utilize different, more local cues for the final decision to deposit eggs. Once females have 'settled' on a prospective oviposition site, signals that they employ to decide whether to oviposit appear to be located on the ovipositor (Takamura & Fuyama 1980; Chess & Ringo 1985). Taste receptors also are present on the proboscis, wings and legs (Stocker 1994; Chyb 2004) and these show sex specific responses (Meunier, Ferveur & Marion-Poll 2000), such that these other taste receptors also may be used in oviposition.

Species differences in oviposition site utilization must originate in intraspecific variation. Considerable intraspecific variability exists for oviposition site preference. For example, *D. melanogaster* rapidly respond to selection for oviposition site preference (Takamura & Fuyama 1977; Bird & Semionoff 1986; Ruiz-Dubreuil & del Solar 1986). Barker & Starmer (1999) and Barker *et al*. (1986), and Barker, Starmer & Fogleman (1994) demonstrated that in natural populations of the *Opuntia*-breeding *D. buzzatii*, there is high heritability for oviposition site based upon the composition of the yeast communities presented to the females. In nature, microbial communities vary among patches of necrotic opuntia, resulting in the maintenance of intraspecific genetic variation for oviposition site. *Drosophila tripunctata*, a species that uses both mushrooms and various fruits, exhibits genetic variation both for settling behaviour and for subsequent oviposition, supporting the complex nature of oviposition site selection (Jaenike 1985). Fanara & Hasson (2001) report a similar finding for *D. buzzatii* and *D. koepferi*.

Depending upon the species and its reproductive ecology, ovipositing females have different patterns of egg laying (Kambysellis & Heed 1971). In some species, females insert the eggs deep into the substrate, while in others the egg is positioned on the surface. Morphological correlates of these behaviours are found in the long chorionic (respiratory) filaments: eggs in species in which eggs are completely inserted into the substrate have long filaments while eggs of species ovipositing on surfaces may lack filaments completely (Kambysellis & Heed 1971). In some species, females lay aggregates of eggs while in other species females oviposit individual eggs seemingly randomly (del Solar & Palomino 1968; Ruiz-Dubreuil & Kohler 1994). Physiological mechanisms

of oogenesis may underlie this difference. In some species, such as the cactophilic *D. mulleri*, oogenesis is synchronous among ovarioles and eggs are matured in clutches as opposed to species like *D. melanogaster*, in which oocytes in each ovariole are at different developmental stages (Kambysellis 1968).

'Functional' ecology

A notable feature of *Drosophila* reproductive ecology is the diversity of resources utilized by different species (Fig. 2). These resources, because of their varying spatial, temporal, microbial, and chemical properties, pose a range of different challenges for resident species. The *Drosophila* model offers an unique opportunity to address how ecology shapes the physiological, behavioural and morphological diversity we observe. Phylogenetic relationships and ecological affinities of many species are well-defined. The availability of full genome sequences of ecologically diverse species (Drosophila 12 Genome Consortium 2007) and precise gene expression patterns in *D. melanogaster* adults and larvae (Fishilevich & Vosshall 2005; Vosshall & Stocker 2007; Laissue & Vosshall 2008; Louis, Piccinotti & Vosshall 2008) allow these questions to be addressed with a degree of sophistication not yet possible for other taxa. For most *Drosophila*, their resources are adult feeding sites, as well as locations to find mates and to oviposit. We can examine not only coarse level phenotypic correlates of resource attributes with reproductive biology, but their functional, evolutionary and genomic foundations also can be probed.

For example, flies identify their breeding sites and their mates via the neurobiological processes that underlie the phenomenon of recognition. Chemoreception involves structures such as neurons and their sensillae and members of the large group of chemoreceptor genes (Robertson, Warr & Carlson 2003) that includes gustatory (Gr), olfactory receptor (Or), and odorant binding (Ob) gene families. Olfactory receptors are associated with the antennae, while gustatory receptors are located in the proboscis, legs, wings, and ovipositor. The two components of oviposition site selection described by Jaenike (1985), settling and oviposition, may well be governed by these gene families. Several studies have examined the evolution of the Or and Gr genes between the specialist species *D. sechellia* and its generalist relative *D. simulans* (Dekker *et al*. 2006; McBride 2007). The numbers of genes in both families are greatly reduced in *D. sechellia* (McBride 2007). Recent studies coupling expression arrays and quantitative PCR (Kopp *et al*. 2008), have indicated that evolution of Or and Ob genes are accelerated in *D. sechellia*, both relative to other genes in the *D. sechellia* genome and to homologous loci in *D. simulans*. Further study of Or, Gr, and Ob genes, directed at sister group comparisons between generalists and specialists or at examining large adaptive radiations (e.g. cactophilic and mycophagous *Drosophila*, Hawaiian *Drosophila*), will help refine the link between ecology, larval and adult behaviour, and gene expression patterns (Stensmyr *et al*. 2003, Guo & Kim 2007; Nozawa & Nei 2007).

Connecting the processes of mate and oviposition site location to larval development is an often-assumed relationship between preference and performance (Rausher 1983). Females are expected to prefer oviposition sites in which their offspring will perform well. Indeed, among the major challenges posed for *Drosophila* larvae by their resources are their chemical constituents and the communities of microbes, parasites and other pathogens found there. This clearly is the case with the toxicity of amanatins in mushrooms (Jaenike *et al*. 1983) and the triterpenes and alkaloids in cacti (Kircher 1982), and bacteria (Foster & Fogleman 1993; Corby-Harris *et al*. 2007). Functional genomic tools provide sensitive indicators of genes whose expression changes in response to both seemingly benign (Carsten, Watts & Markow 2005) and toxic (Matzkin *et al*. 2006) dietary shifts. Here, too, large gene families such as the cytochrome P450s (Danielson, Frank & Fogleman 1994) and glutathione S-transferases (Matzkin 2008) show evidence of major evolutionary changes corresponding to host use. In the case of the glutathione S-transferases, the surface has only begun to be scratched with respect to associating particular host chemicals with the pathways used by the flies to metabolize them. No evidence for genetic correlations between components of female oviposition site selection and larval performance has been identified (Jaenike 1989). If loci underlying host use and loci underlying oviposition site preferences can be identified using genomic approaches, however, the opportunity to look for patterns such as linkage disequilibrium may reveal previously hidden associations. The 12 sequenced *Drosophila* genomes have the potential to identify candidate loci for these associations. With respect to host location and preference, members of the Or and Gr gene families mentioned above are obvious candidates. In terms of performance on different hosts, the sequenced genomes of 12 *Drosophila* species reveal patterns of evolution at glutathione S-transferase loci suggestive of niche- specific selective patterns (Low *et al*. 2007). Further study on these candidate loci, as well as others (Kopp *et al*. 2008), are the first step towards a functional genomic understanding of ecological performance and preference.

Genes involved in immune responses will be especially attractive and relevant targets for future studies. For example, by virtue of the nature of their breeding sites, larvae and adults encounter a wide range of microbes. Interestingly, of over 200 *Drosophila* species screened, only two endosymbionts, *Wolbachia* and *Spiroplasma*, have been found to invade these flies (Mateos *et al*. 2006), and most flies are free of any infection. It is thus no surprise that immune system genes are rapidly activated by microbes in the *Drosophila* larval gut (Bischoff *et al*. 2006; Senger, Harris & Levine 2006), and that immunity genes have been found to experience rapid turnover and positive selection in the 12 *Drosophila* species sequenced to date (Clark *et al*. 2007; Sackton *et al*. 2007).

Spatial and temporal features of breeding site distribution for different *Drosophila* species clearly vary widely but are not yet well-quantified for most species. Surveying *Drosophila* breeding site distributions is a straight-forward activity that can provide the basis for testing many evolutionary ecological hypotheses, for example the relationship between habitat

continuity and population genetic structure (Shoemaker & Jaenike 1997; Hurtado *et al*. 2004) Traits like resistance to environmental stress and dispersal ability are expected to be related to distances travelled between breeding sites (Stratman & Markow 1998).

Functional ecology represents the true crossroads between ecological patterns and the processes and mechanisms that underlie them. Above we have seen an overview of the reproductive biology of *Drosophila* as it relates to their ecology. Several ecological aspects of successful reproduction stand out as promising candidates for genomic approaches to understanding their diversification. While *Drosophila* typically have been viewed primarily as a genetic model organism, they represent, as well, an ecological model organism and one that brings the power of genetic and genomic tools to bear on ecological questions.

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