

REVIEW

Phylogeny, environment and sexual communication across the *Drosophila* genus

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ABSTRACT

Social behaviour emerges from the local environment but is constrained by the animal's life history and its evolutionary lineage. In this perspective, we consider the genus *Drosophila* and provide an overview of how these constraints can shape how individuals interact. Our focus is restricted to visual and chemical signals and how their use varies across species during courtship – currently the only social behaviour well-studied across many *Drosophila* species. We broadly categorize species into four climatic groups – cosmopolitan, tropical, temperate and arid – which serve as discussion points as we review comparative behavioural and physiological studies and relate them to the abiotic conditions of a species environment. We discuss how the physiological and behavioural differences among many fly species may reflect life history differences as much as, or even more than, differences in phylogeny. This perspective serves not only to summarize what has been studied across drosophilids, but also to identify questions and outline gaps in the literature worth pursuing for progressing the understanding of behavioural evolution in *Drosophila*.

KEY WORDS: Drosophilid, Life history, Social communication

Introduction

Throughout the 20th century, laboratory studies on the fruit fly *Drosophila melanogaster* have progressed our understanding of mechanisms surrounding development, genetic heredity, neuronal circuitry and social behaviour. This ever-increasing bounty of knowledge gained from *D. melanogaster* within the laboratory as a model organism can obscure interest in its evolutionary history. For example, teasing apart the neuronal circuits and genes mediating mating behaviour does not rely on appreciating that *D. melanogaster* originates in Africa and only increased its geographic range with human activity thousands of years ago (David and Capy, 1988). Despite our history together, it has been only within the past dozen years that we have begun to appreciate the social life of *D. melanogaster*. Various studies have demonstrated that *D. melanogaster* displays distinct courtship/mating behaviour, aggressive behaviour, social learning and emergent group-based behaviour (Billeter and Levine, 2013; Schneider et al., 2012a). These behaviours are thought to be mediated via sight (Agrawal et al., 2014; Kohatsu and Yamamoto, 2015), taste (Schneider et al., 2012b), touch (Schneider et al., 2012b; Ramdya et al., 2015) and olfaction (Duménil et al., 2016). Yet *D. melanogaster* is simply one species nested within the approximately 1500 classified species of the *Drosophila* genus (Markow and O'Grady, 2006). Upon

considering the diversity of drosophilids, a critical question becomes evident: what is '*Drosophila*' social communication? How much of it is shared, and how much of it is unique to a particular species within its niche?

There are many examples of striking behaviours observed contrasting those of *D. melanogaster*. The Hawaiian *Drosophila* species, with their conspicuously bright colouration and territoriality during mating, have evolved complex courtship rituals (Spieth, 1974). Cactophilic species also differ in their mating behaviour from *D. melanogaster* in that flies mate away from feeding sites (Markow, 1988), and females produce courtship song duets with males (O'Grady and Markow, 2012). Male flies of species within the *obscura* group have been observed to regurgitate a nutritious pre-nuptial gift for the female to consume during courtship (Steele, 1986). Across all the aforementioned taxonomic groups, many species have been reported to be unable to mate in the dark (Grossfield, 1971) and to vary in mating frequency (Markow, 2002). One possible explanation for such varied behaviours is the extreme abiotic factors that characterize some of these species' niches. For instance, cactophiles such as *D. mojavensis* and other *repleta* species endure temperatures ranging from below 5°C at night to over 40°C during the day (Gibbs et al., 2003). As a result, it is likely that these cactophilic species evolved physiological tolerance in response to these extreme abiotic factors. An example of a physical trait that confers resistance to these extreme conditions are the cuticular lipids [also known as cuticular hydrocarbons (CHCs)], which could also impact the behaviour of these species.

Although comparative studies of behaviour and social signalling are scarce, a wealth of comparative physiological studies across *Drosophila* species have characterized their responses to abiotic stresses, such as heat (Stratman and Markow, 1998; Krebs and Loeschcke, 1995; Loeschcke et al., 1994; Hoffmann et al., 2003; Kellermann et al., 2012b), cold (David et al., 1998; Gibert and Huey, 2001; Kellermann et al., 2012a; Andersen et al., 2015; Gibert et al., 2001; Bertoli et al., 2010) and desiccation (Gibbs and Matzkin, 2001; Kellermann et al., 2012a; Matzkin et al., 2009; Gibbs and Markow, 2001; Kalra et al., 2014; Hoffmann and Harshman, 1999). Not surprisingly, abiotic stress tolerances have been reported to be reliable indicators of a species' geographic distribution (Kellermann et al., 2012b; Andersen et al., 2015). This geographic correlation to stress tolerance is strengthened through comparative studies that examined stress resistance across many species. Using phylogenetic comparative methods, these studies outlined the extent to which convergent evolution and common descent influences the evolution of stress resistance (Kellermann et al., 2012a,b). Another aspect that arises out of these comparative studies, and supported by established climate models (Peel et al., 2007), is an attractive generalization that 'arid species' possess high abiotic stress tolerance, 'tropical species' possess low abiotic stress tolerance and 'temperate species' are intermediate. The exceptions are species known to have world-wide distributions, which are often

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referred to as ‘cosmopolitan species’. Examples of these include *D. melanogaster*, *D. simulans*, *D. hydei*, *D. funebris* and *D. immigrans* (Markow, 2015). While these categories represent over-simplified generalizations, they serve as discussion points to highlight that environmental factors, such as climate and precipitation patterns, may constrain the evolution of social behaviour.

Social communication in *Drosophila* involves a complex combination of visual, auditory, mechanosensory and chemosensory cues (Bontonou and Wicker-Thomas, 2014). The role of these sensory modalities and their neurological input and output mechanisms are best known in *D. melanogaster* because of the organism’s well-characterized genome and abundance of genetic tools for neurological manipulation. For this Review, we are interested in elaborating on aspects of social communication beyond this single species. We are interested in elucidating the extent to which diverse *Drosophila* species vary in their social behaviours and how phylogeny and climatic factors influence these behavioural differences. What is a species-specific behaviour? Given their ecological range, what individual adaptations do these species have? Would such adaptations engender differences in their behaviour? How do these behavioural differences map onto the *Drosophila* phylogeny? Is there evidence of convergent behaviours? As we will discuss, these topics are under-represented in the *Drosophila* literature. Here, we review studies that have shed light on how visual and chemical communication displayed during courtship/mating varies across species. We choose to focus on these two sensory modalities because they play important roles within the larger context of social communication of *D. melanogaster* (Agrawal et al., 2014). We limit the context of social communication to courtship/mating because it is the only social behaviour studied across a wide variety of species. Throughout this Review, we will focus on a small subset of the overall diversity of the *Drosophila* genus and, whenever possible, connect behavioural differences to phylogeny and distribution patterns (i.e. tropical, temperate, arid and cosmopolitan; see Fig. 1).

Visual communication

Visual perception of *D. melanogaster*, brought by their compound eyes composed of photoreceptive ommatidia individually connected to their optic lobes, provides the fly with a wide range of vision essential for flight, but limits colour and object recognition (Borst, 2014). However, some studies have shown that the limited object recognition *D. melanogaster* possesses is sufficient for social mate choice learning (e.g. Mery et al., 2009). It has also been demonstrated that this species prefers to mate in the dark (Hardeland, 1972), suggesting that visual cues are not essential for mate communication. However, this is not the case throughout the *Drosophila* genus.

Since the 1940s, discrepancies were being documented between *Drosophila* species on their visual communication during mating. *Drosophila subobscura*, for example, will not mate in the absence of light, which contrasted with the behaviour of the closely related species *D. pseudoobscura* and *D. persimilis* (Wallace and Dobzhansky, 1946). Indeed, observations of the courtship sequence of *D. subobscura* involve a ‘wing-dance’ where the male faces the female and side-steps while spreading his wings (Pinsker and Doschek, 1979). Wing removal in *D. subobscura* has been demonstrated to have a detrimental effect on copulatory behaviours in males (Grossfield, 1968). This provides evidence of the wing-dance serving as a crucial visual cue during courtship, as *D. subobscura* has been reported to not produce courtship song through wing vibration (Ewing and Bennet-Clark, 1968).

Here, we compiled a list of 47 species and noted their ability to mate in the dark. Most of this list is attributed to Grossfield (1971), who categorized 42 species into three classes: (Class I) light independent, meaning the species can mate in the dark; (Class II) facultative dark mating, meaning darkness has a slight repressive effect on mating; and (Class III) dark repressed, meaning the species cannot mate in the dark (Grossfield, 1971). By displaying a phylogeny of species and mapping their ability to mate in the dark on the tree, we see that most species rely on the presence of light in order to successfully mate (Fig. 2). This suggests, based on our limited sample, that diurnal mating may be the ancestral trait of all *Drosophila* species. We see evidence of conserved traits across closely related species, as the tropical *immigrans* group species are all Class III species (Fig. 2). Also, the *quinaria* species mostly share the inability to mate in the dark (Fig. 2). On the other end of this behavioural spectrum, the ability to mate in the dark appears to have evolved multiple times, and we note that ‘cosmopolitan’ species such as *D. melanogaster*, *D. hydei*, *D. mercatorum*, *D. immigrans*, *D. funebris* and *D. ananassae* are not closely related, yet they freely mate in the dark. It is conceivable that these species, being human commensals (Markow, 2015; Markow and O’Grady, 2008), thrive in dark microhabitats created by humans and such environments might have selected for visually optional mate communication.

Although some of the dark-repressed class III species, such as *D. subobscura* and the *quinaria* species, are distributed in temperate regions of the world, 11 out of 21 Class III species are distributed in tropical regions (Figs 1 and 2). This is consistent with the observation that many tropical species exhibit colourful morphologies and visually display stereotyped courtship rituals. The Hawaiian picture wing species are a classic example of *Drosophila* that depend on visual cues during courtship. These species show diversity in wing pattern colours, and display clear sexual dimorphism based on their morphology (Edwards et al., 2007). The diverse wing patterns on the male flies are thought to offer species-specific visual cues to the female during courtship (Spieth, 1974). Similarly, *D. grimshawi*, a Hawaiian representative, cannot mate in the dark and is also a Class III species (Grossfield, 1971). This complements accounts of Hawaiian courtship involving males securing a territorial lek and facing females during a complex visual-based courtship ritual (Spieth, 1974).

Yet wing patterns in *Drosophila* are not traits restricted to the tropical Hawaiian clade, as similar characteristics are observed in other distantly related tropical species. A comparative study investigating sexual characters in a variety of *melanogaster* species found throughout Australasia (referred to as ‘Oriental’ species; Fig. 1) has revealed that the dark spots found on the wings of *D. eugracilis*, *D. suzukii*, *D. pseudotakahashii*, *D. fuyamai*, *D. biarmipes* and *D. elegans* have independent evolutionary origins (Kopp and True, 2002). It is thought that these wing spots serve as a crucial visual cue during the courtship of these species (Fuyama, 1979). Given that the Hawaiian and some of the ‘Oriental’ species are tropical, and that they have evolved melanised wing spots independently, one hypothesis is that the common abiotic conditions of tropical environments in different areas of the world play a role in shaping these convergent traits. Perhaps tropical conditions with stable climates relax naturally selective pressures on *Drosophila* and enable rapid evolutionary changes to behaviour through sexually selective mechanisms, resulting in convergence. Another possibility is that the relative density and variety of species in tropical regions require mate coordination that can communicate precisely at short distances without being influenced by surrounding messages (as could be the case with chemical signalling). However, out of the ‘Oriental’ species mentioned with the wing spots, there is

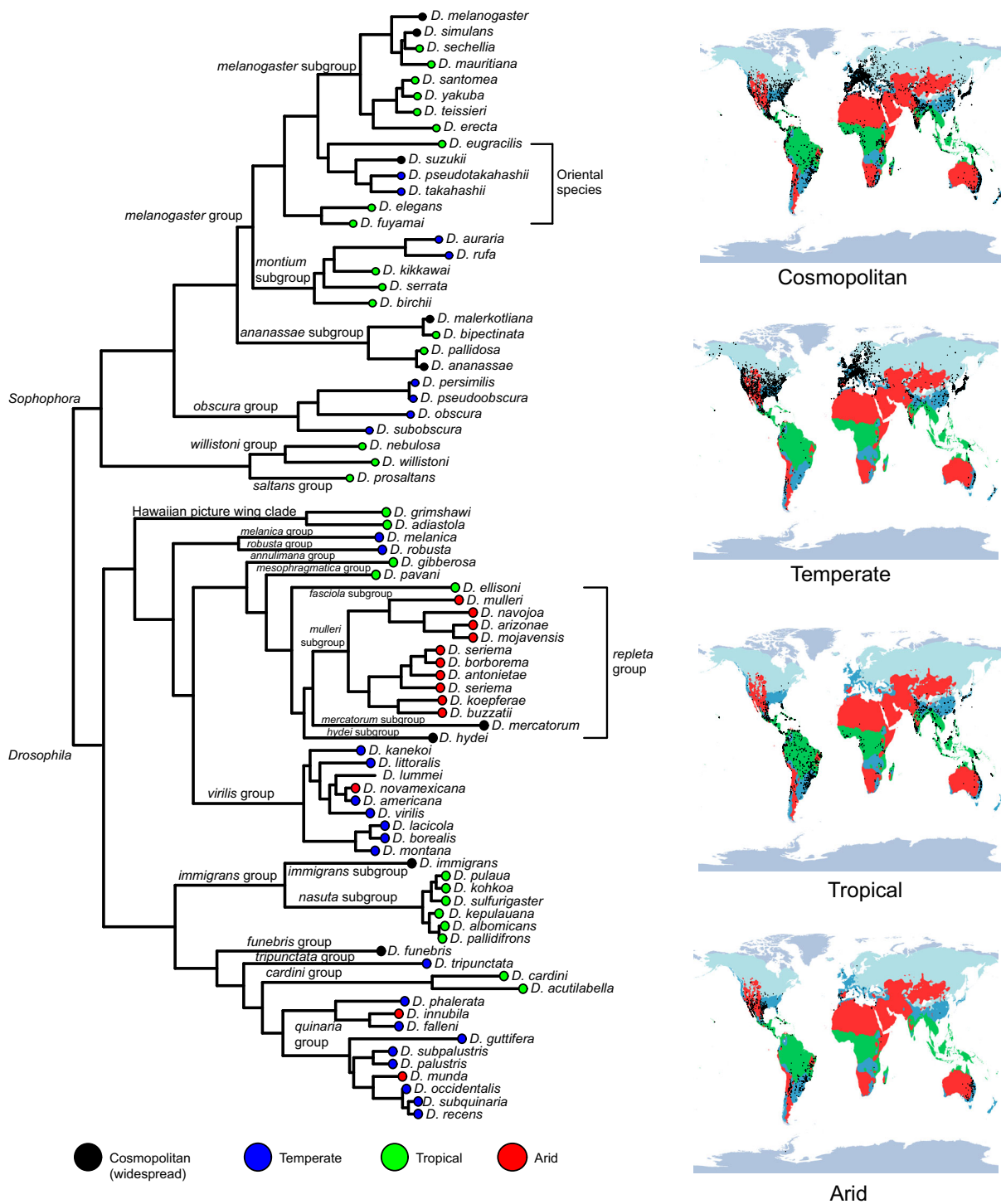


Fig. 1. Generalized relationship between habitat distributions and phylogenetic relationships across a variety of *Drosophila* species. Phylogeny is modified from van der Linde et al. (2010) for illustrative purposes. Species absent from van der Linde et al. (2010) were manually added based on molecular phylogenies from other sources (Kopp and True, 2002; Yu et al., 1999; Spicer and Jaenike, 1996; Perlman et al., 2003; Spicer and Bell, 2002). The distributions of species are mapped in four 'climatic categories' that summarizes their range the best. Each species was categorically defined based on a combination of sources (see Table 1). The world maps displaying the distribution of each species are subdivided and colour coded into 'temperate' (blue), 'tropical' (green) and 'arid' (red) regions based on published climate data (Peel et al., 2007). All points on the world maps are GPS coordinates of documented collection sites (acquired from <http://www.taxodros.uzh.ch/>). The distribution of each species based on these GPS coordinates is in agreement with published distribution maps (Markow and O'Grady, 2006).

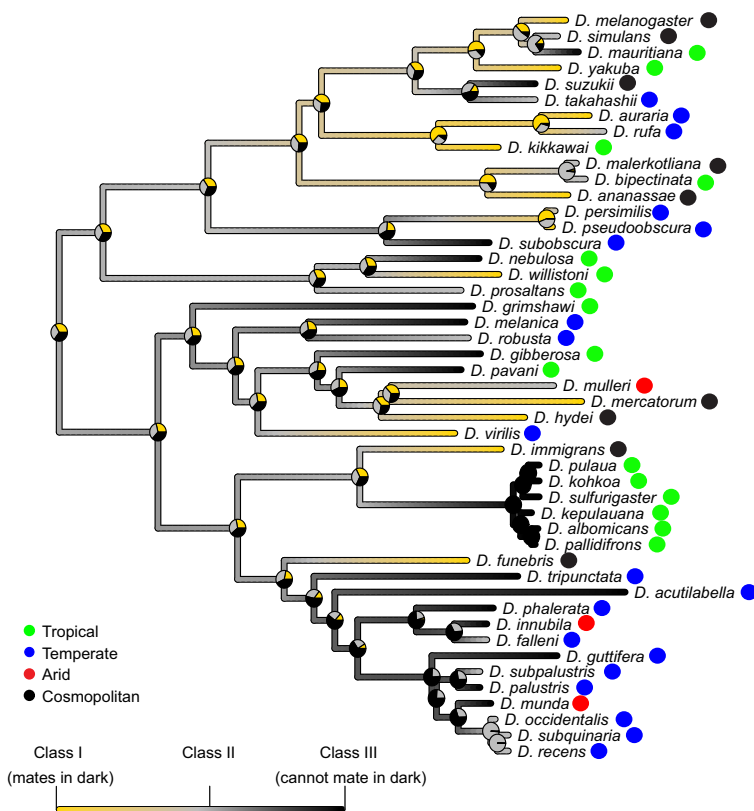


Fig. 2. Mapping light-dependence on courtship/mating behaviour on a phylogeny of 47 species pruned from Fig. 1. Gold branches represent species able to mate in the dark (Class I), grey branches represent species with a small mating suppression in the dark (Class II) and black branches represent species that cannot mate in the dark (Class III). Pie charts on each internal node represents the probabilities of the three possible ancestral states (Class I, Class II or Class III) based on the outcome of 1000 simulated stochastic character maps through the ‘make.simmap’ function in R (phytools package). The coloured shading across the branches was executed through the ‘contMap’ function in R (phytools package) to help visualize potential trait changes across internal nodes of the phylogeny. The coloured ellipses next to each species name are the colour-coded climatic categories defined in Fig. 1.

only evidence of *D. suzukii* displaying courtship repression in the dark (Grossfield, 1971). To gather support for such hypotheses, testing the courtship repression of a larger sample of tropical ‘Oriental’ species with and without melanised wing spots would be a good start.

Unlike the tropical species with sexually dimorphic wing spots, *D. mojavensis* males and females look alike and during courtship the males mostly position themselves behind the female (Markow and O’Grady, 2005; O’Grady and Markow, 2012; Krebs and Bean, 1991). This lack of sexual dimorphism is seen in other *virilis* and *repleta* species, such as *D. virilis* (temperate) and *D. hydei* (cosmopolitan), which are examples of two species that freely mate in the dark (Grossfield, 1971). Although the arid cactophiles – *D. mojavensis*, *D. arizonae* and *D. buzzatii* – are well studied, we are not aware of any experiments that directly tested the ability of these species to mate in the dark, although the close relatives of these species, *D. mulleri* (arid) and *D. mercatorum* (cosmopolitan), are Class II and Class I species, respectively (Grossfield, 1971; Ikeda, 1976). In addition, locomotor activity experiments revealed that, in a laboratory setting, *D. mojavensis* displays peak activity during dawn and dusk (Hardeland and Stange, 1973). Its activity cycle may have evolved in response to the stress of nocturnal cold temperatures, which may inhibit nocturnal activity and mating, combined with the aridity and heat of the day. These two factors are avoided by scheduling activity during transition times, as is seen in *D. mojavensis*, which mates predominately after sunrise (Krebs and Bean, 1991). This could also be true in the Sonoran Desert endemic species, *D. nigrospiracula* and *D. pachea*, that, like *D. mojavensis*, tolerate desiccation stress and temperatures that may exceed 40°C during the day and between 0 and 5°C at night (Gibbs et al., 2003). Experiments that screen a large sample of arid species for their ability to copulate in the dark would offer insight into our speculation that the arid climates influenced the evolution of

mating activity during dawn, when temperatures in these desert environments are neither too hot nor too cold. Consequently, this may have relinquished visual signalling during courtship, and perhaps other social behaviours because, at this time of day, there may be lower sun exposure on cactus necroses.

Out of the 47 species we illustrate in Fig. 2, the majority of species possess either a slight repression of courtship activity (Class II) or complete repression of courtship activity (Class III) when maintained in the absence of light. The majority of cosmopolitan species are Class I (5 out of 8), the majority of temperate species are at least slightly repressed in the dark (9 out of 20 are Class II, 7 out of 20 are Class III), tropical species are mostly Class III (11 out of 16; Fig. 2) and while the three arid species are mostly Class III (2 out of 3), although only one of these species is nested within the *repleta* group. Even if the phylogeny and ecology are sometimes correlated (such as the tropical *nasuta* cluster), we see that light-dependent courtship is a complex trait that appears to have been shaped by both phylogenetic and ecological pressures. Species within similar ecologies share mating habits with regards to the light, which may outline some aspect of environmental influence on the dependence of visual cues for social communication. Unfortunately, this sample of 47 species does not offer an accurate reflection of the approximately 1500 *Drosophila* species. Any patterns regarding the distribution, phylogeny and emphasis of visual cues in these species could result from sampling bias. To complicate matters, the timing of mating events varies across species (Hardeland, 1972) and across populations of the same species (Billeter et al., 2012). With this in mind, it would be beneficial to examine whether populations of the same species sampled across the globe are all classified together. This would enable one to directly test associations between environment and visual-dependent courtship communication. One hypothesis is that a species broadly categorized as dark-repressed (Class III), but collected from a more arid and warm region, could

exhibit mating behaviours more similar to Class II species in its attempts to thermo-regulate and avoid activity during peak temperatures. It also seems worthwhile to identify trends in the phylogeny regarding light-dependent mating to highlight exceptions – these exceptional cases tentatively hint at ecological niches that have spurned adaptation. One such example is *D. willistoni* and *D. nebulosa*. These are two related tropical species, and one can mate in the dark (*D. willistoni*) while the other cannot (*D. nebulosa*; see Fig. 2). The life history that caused this divergence is an open question.

Chemical communication

In all *Drosophila* species, volatile pheromones and non-volatile cuticular lipids are involved in many aspects of social interactions. The synthesis of these chemical compounds is quite plastic and depends on temperature (Savarit and Ferveur, 2002), humidity (Frentiu and Chenoweth, 2010), photoperiod and social environment (Krupp et al., 2008). It has been observed across many species that during courtship males tap the abdomen of females (Pinsker and Doschek, 1979; Spieth, 1974), and this behaviour has also been observed in groups of flies of the same sex (Schneider et al., 2012b; Ramdya et al., 2015; Sexton and Stalker, 1961). This tapping behaviour is assumed to involve the fly tasting chemical secretions in gustatory receptors on their forelegs (Vosshall and Stocker, 2007), though purely tactile information can also be conveyed (Ramdya et al., 2015). The chemical compounds that are thought to be exchanged during these tapping behaviours are the CHCs that are synthesized in the oenocytes (Billeter et al., 2009). Extracting the CHCs of a fly may purify a blend of compounds that vary quantitatively and qualitatively based on the length of the molecule in carbons, the saturation and branching.

Across species, CHCs are known to vary in quantity. For instance, the arid *virilis-repleta* species tend to synthesize higher quantities than *Sophophora* and other tropical species. *Drosophila mojavensis* has been reported to contain two orders of magnitude more CHC extract in its cuticle compared with *D. melanogaster* (Ferveur, 2005). The high quantity of CHCs in species adapted to drier environments and the latitudinal variation of CHCs within *D. melanogaster* (Frentiu and Chenoweth, 2010) suggest that CHCs also play a role in desiccation resistance. Contrary to CHC quantity, the length of CHC chains shows more conservation across species groups: Hawaiian and *Sophophora* species produce the shortest CHCs (between 23 and 29 carbons in most species), the *virilis* species produce CHCs that are slightly longer (between 22 and 31 carbons), and the *repleta* species synthesize the longest (between 28 and 40 carbons; Bontonou and Wicker-Thomas, 2014). We compiled a list of known CHC compounds synthesized in 36 species and computed the average length of CHCs (based on the number of carbons) to help visualize any climatic correlation of CHC differences across *Drosophila* (Fig. 3). When incorporating a variety of climatic variables (see Fig. 3), we see that environment predicts average CHC length to a reasonable degree across all environments (Fig. 3A), as well as within environments (Fig. 3B–D). Temperate and tropical species overlap in the CHCs they produce, whereas arid species cluster on their own (Fig. 3). Two conclusions of our preliminary regressions are worth mentioning as they deserve further investigation. One is that the temperature of the warmest month has a negative effect on CHC length (at least in a combined model or temperate species on their own). If (and potentially why) large CHCs are detrimental in temperate species that experience extreme warm months is an

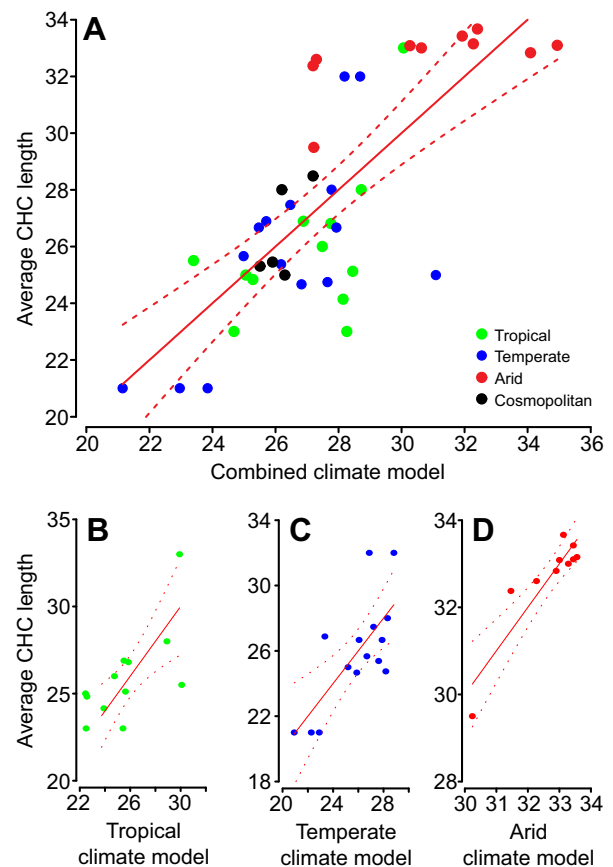


Fig. 3. Climate, especially precipitation, is a reliable predictor of the average cuticular hydrocarbon (CHC) length across *Drosophila*. Climate data (predictive of mid-Holocene obtained from worldclim.org) were averaged across all collection sites known for each species (obtained from www.taxodros.uzh.ch). A step-wise linear regression was used on climate variables. (A) Across all species for which data were available, the final model ($P < 0.001$, adj. $r^2 = 0.498$) included five predictors and six terms: average CHC length = $21.52 - 0.69 \times [\text{temperature } (^{\circ}\text{C}) \text{ of warmest month}] - 0.19 \times [\text{precipitation (mm) of wettest month}] + 0.41 \times [\text{precipitation (mm) of driest month}] + 0.47 \times [\text{coefficient of variation of precipitation}] + 0.07 \times [\text{precipitation (mm) of wettest quarter}] - 0.01 \times [\text{coefficient of variation of precipitation} \times \text{precipitation (mm) of driest month}]$. (B) The tropical species model ($P = 0.005$, adj. $r^2 = 0.523$) only included a single predictor: average CHC length = $22.15 + 0.02 \times [\text{precipitation of driest quarter (mm)}]$, which implies CHC length increases with measures of precipitation. (C) Temperate species were modelled ($P = 0.002$, adj. $r^2 = 0.49$) by a single predictor: average CHC length = $46.26 - 0.74 \times [\text{temperature } (^{\circ}\text{C}) \text{ of warmest month}]$, where warmer extremes lead to shorter CHC lengths. (D) The model of arid species' CHC length ($P < 0.001$, adj. $r^2 = 0.80$) only included a single predictor: average CHC length = $33.82 - 0.7 \times [\text{precipitation (mm) of the driest month}]$. Cosmopolitan species' CHC length was not significantly related to any climate variables (not shown). The coefficient of variation is the standard deviation of the monthly precipitation as a percentage of the annual precipitation. Climate variables not included in the linear regressions were: mean annual temperature, annual precipitation, average diurnal range in temperature, average diurnal range/annual range, annual range in temperature, coefficient of variation in temperature, temperature of the coldest month, temperature of the coldest, warmest, driest, and wettest quarter, and precipitation of the coldest and warmest quarter.

interesting question. Another general conclusion is that precipitation can have different effects on tropical (positive) or arid (negative) species. This supports the notion that it is not simply the measured regional abiotic factors, but potentially interactions with complex microhabitats that shape *Drosophila*'s physiology. The long

hydrocarbon chains observed in arid *repleta* species likely correlates with their increased thermal and desiccation resistance (Gibbs and Matzkin, 2001; Kellermann et al., 2012a; Matzkin et al., 2009). Both thermal and desiccation tolerance have shown phylogenetic correlation (Kellermann et al., 2012a,b), and the long CHC chain length exclusive to *repleta* species are intuitively very similar in pattern. Perhaps the common ancestor of the *repleta* radiation acquired the ability to polymerize elongated cuticular hydrocarbons and this, in turn, enabled these species to thrive in arid climates. This speculation is an example of how traits that are physiological adaptations to abiotic stresses can also play a role in social communication. Whether specific CHCs in these arid *repleta* species influence mating behaviour, much like how 7,11-heptacosadiene stimulates courtship behaviour in male *D. melanogaster* (Billeter et al., 2009), is currently not well understood.

Species differences in CHCs are not only correlated to abiotic selection pressures. Even the particular blends of cuticular hydrocarbons appear to be a trait that expresses species identification signals that have higher variation between closely related species, suggesting a link to reinforcement barriers. A classic example is *D. melanogaster* and *D. simulans*, two sibling species that often coexist in sympatry (Markow, 2015). *Drosophila melanogaster* females produce unsaturated CHCs with two double bonds in the carbon chain (dienes) and the males do not, making this species sexually dimorphic in terms of its CHC blends. However, *D. simulans* does not produce dienes, and dienes exhibit anti-aphrodisiac qualities to *D. simulans* males (Billeter et al., 2009). Considering the CHC profile of other closely related species reveals that *D. sechellia* and *D. erecta* females produce dienes like *D. melanogaster*, but *D. yakuba* females do not (Ferveur, 2005; Cobb and Jallon, 1990). *Drosophila yakuba* and *D. erecta* are sibling species that are sympatric in western Africa (Lachaise et al., 1988), and this parallels the relationship between *D. melanogaster* and *D. simulans*, suggesting character displacement. Although the size and quantity of CHCs a species synthesizes could depend on environmental pressures, such as heat and aridity, the qualitative aspects of CHC profiles (i.e. the range of compounds synthesized) appear sensitive to reproductive isolation mechanisms. Interestingly, an experimental evolution study utilizing *D. serrata* and *D. birchii* found that exposing the two species to novel diets (rice and corn) caused a divergence in both CHCs and female mating preference (Rundle et al., 2005). Consequently, the distribution of individual CHC compounds may not hold any phylogenetic correlation and may evolve frequently based on reinforcement, mate selection and shifts in host resources. Accordingly, by compiling a list of species that are known to lack dimorphism in their CHC profile and considering the phylogenetic relationships of these species, we see that this is a trait that appears to have independent origins (Fig. 4). Interestingly, species that show courtship repression in the dark, such as *D. grimshawi*, *D. suzukii*, *D. simulans* and *D. mauritiana*, are examples of species without dimorphic CHCs (Fig. 4). The lack of dimorphism in their CHCs may partially explain why these species depend on visual cues for courtship and mating. Of special note is that the arid species sampled are exclusively dimorphic in CHCs. This adds credence to our earlier hypothesis of arid *repleta* species underemphasizing visual signals during courtship. One hypothesis that follows from this observation is that the environmental pressures on arid species are so high that dimorphic CHC profiles became essential for social communication either in dark and cool microhabitats, or at a time of day when sun exposure is reduced.

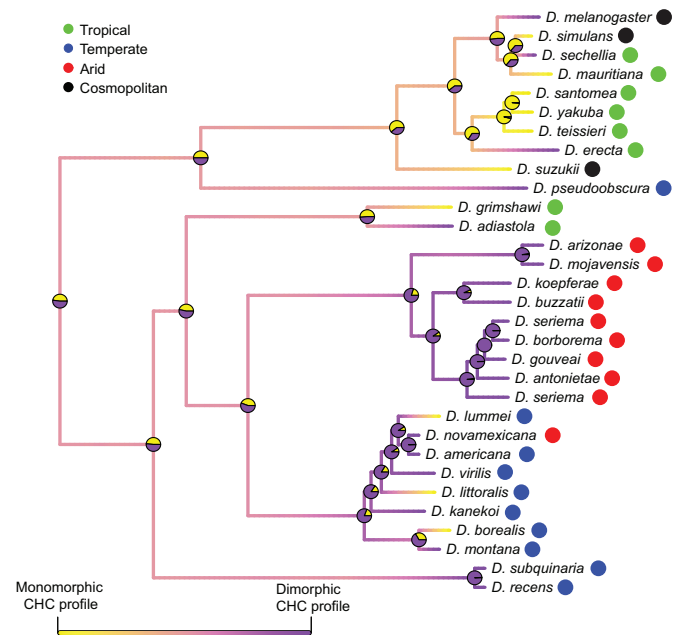


Fig. 4. Mapping CHC dimorphism on a phylogeny of 31 *Drosophila* species pruned from Fig. 1. Colour gradients represent a density map combining the outcome of 1000 stochastically simulated character maps (phytools package). Purple branches represent species with sexually dimorphic CHC profiles, yellow branches represent species with CHC profiles that are not sexually dimorphic and intermediate shades represent regions that transition between the two character states. Each node is labelled with a pie chart illustrating the probability of each of the two possible ancestral states (dimorphic CHC profile, monomorphic CHC profile) based on the ancestral reconstructions of all 1000 stochastic character maps. We designated species as dimorphic if the CHC profile of male and female flies had evidence of statistical differences, or if the two sexes displayed qualitative differences (e.g. males producing compounds females do not produce). The coloured ellipses next to each species name are the colour-coded climatic categories defined in Fig. 1.

Cuticular hydrocarbons serve as chemical signals at short range and are mainly detected through physical contact. In *D. melanogaster*, longer range olfactory communication is known to be carried out through cis-vaccenyl acetate (cVA), which is produced in the ejaculatory bulb of male flies (Butterworth, 1969). This compound is thought to play multiple roles: aggregation (Bartelt et al., 1985), aggression (Vander Meer et al., 1986; Wang and Anderson, 2010) and courtship inhibition (Vander Meer et al., 1986). Males transfer cVA to females during copulation, which then acts as an ‘anti-aphrodisiac’ to other males, and while cVA is in the female reproductive tract it coats the eggs as they are oviposited. *Drosophila melanogaster* females tend to oviposit communally and this significantly affects the survival of larvae because large aggregates of larvae can outcompete harmful microbes, enhancing their survival (Wertheim, 2001; Rohlf, 2005). However, cVA is restricted only to *melanogaster* species and distantly related *immigrans–tripunctata* species (Symonds and Wertheim, 2005). Across other species, pheromone compounds that facilitate aggregation are generally volatile esters, ketones or unsaturated hydrocarbons (Hedlund et al., 1996). Examples of these include ethyl hexanoate, found in *immigrans–tripunctata* species (Symonds and Wertheim, 2005), 10-heptadecanone, found in *repleta* species (Bartelt et al., 1989; Schaner and Jackson, 1992), and heneicosene, found in *virilis* species (Bartelt et al., 1986). Symonds and Wertheim (2005) mapped the known aggregation

pheromones in 28 *Drosophila* species and found a phylogenetic pattern to the distribution of these pheromones across species. While the authors suggested that pheromonal evolution occurred gradually in *Drosophila* (Symonds and Wertheim, 2005), we wonder how many examples of convergent evolution (e.g. cVA in *D. immigrans* and *D. melanogaster*) correlate with the abiotic conditions of the environment they are distributed in.

A recent study demonstrated that species within the *Drosophila* subgenus tend to be sexually dimorphic for a family of triglycerides (TAGs). Interestingly, these TAGs are synthesized in the male ejaculatory bulb of these species and appear to function as anti-aphrodisiacs, much like cVA in *D. melanogaster* (Chin et al., 2014). Expression of these compounds also correlated with the age of reproductive maturity in *D. mojavensis* and *D. arizonae*, suggesting that these compounds are necessary for courtship (Chin et al., 2014). These compounds are highly conserved in the *virilis-repleta* species and completely absent in species within the *Sophophora* subgenus. One hypothesis is that the utilization of TAGs for pheromonal communication may be related to the adaptations of arid and cactophilic *virilis-repleta* species; that is, perhaps they are more stable under high temperatures and low humidity. As a result, TAGs, instead of cVA, may have evolved in these species as a result of being more effective for chemical communication. Another recent study described a novel pheromone called CH503, which is only expressed in the *melanogaster* subgroup species, yet appears to act as another anti-aphrodisiac pheromone in a variety of species (Ng et al., 2014). In fact, species in the *Drosophila* subgenus that do not express this compound, such as *D. virilis* and *D. mojavensis*, displayed a stronger courtship suppression response to females perfumed with this compound than did species that express this compound (Ng et al., 2014). This suggests that there is conservation in the response to pheromones across species in the subgenera *Drosophila* and *Sophophora*. The synthesis of pheromone profiles may vary across species, but their receptors may display conservation across species.

We have come across several open issues relating to CHCs across species. Although CHC quantity appears somewhat correlated with aridity, climate seems to predict CHC length and arid species strongly cluster together (Fig. 3). Untangling reproductive barriers, ecology and phylogeny in CHC blends is an ongoing field of study. There are also several tantalizing aspects that appear mysterious, including cVA as a convergent aggregation pheromone, and the general response to the species-specific hydrocarbon CH503. More studies dedicated to pheromone compounds and their behavioural effects in other *Drosophila* species might lead to a broader understanding of the evolution of social behaviour in *Drosophila*. One issue that keeps returning is the lack of a comprehensive database in which to catalogue collection sites and hydrocarbon profiles. This is sorely needed, as the blend of CHCs that a species produces can vary with latitude (Frentiu and Chenoweth, 2010). This variation across populations of the same species can reveal greater insights into the diversity and correlations between CHC blends and the environment. It is likely that pheromone evolution across *Drosophila* species is influenced by a combination of life history factors (such as host resource preference, temperature tolerance, etc.) and phylogenetic history. Disentangling each factor promises to be an exciting and rewarding field of study.

Broader perspectives and conclusions

Compared with *D. melanogaster*, the sensory modalities of social communication are less thoroughly studied in other species. Studies that do focus on the social behaviour of other *Drosophila* species

tend to focus on those with striking ecologies, such as the Hawaiian species (Spieth, 1974; Edwards et al., 2007; Widemo and Johansson, 2006) and the cactophilic species (Markow, 1988; Krebs and Markow, 1989; Krebs and Bean, 1991; Chin et al., 2014), or on species that coexist in sympatry to study reinforcement mechanisms (Noor et al., 2001; Noor and Coyne, 1996; Markow, 1981; Civetta and Cantor, 2003; Giglio and Dyer, 2013; Gleason et al., 2012). While valuable, these studies often only examine select aspects of certain species, which creates gaps between comparative data. To visualize these gaps, in this Review, we compiled qualitative visual and chemical communication data of 75 species (Table 1). Only nine of these species overlap in having been tested for mating activity in the dark and having their CHC profiles examined (Table 1).

Generally speaking, unlike *D. melanogaster*, most species appear to require light and visual cues for successful copulation (Fig. 2). Many of the species that freely copulate in the dark are cosmopolitan (Figs 1,2). Most tropical species, irrespective of their placement in the *Drosophila* phylogeny, appear to depend on visual cues, and this could explain why species found in tropical regions, such as the Hawaiian species, are sexually dimorphic in their abdomen and wing colour morphology (Edwards et al., 2007). Also, some tropical species display stereotyped visual displays during their courtship ritual that involve directly facing the female (Markow and O'Grady, 2005; Spieth, 1974). These visual-based courtship rituals may result from relaxed abiotic selective pressures on tropical species, but enhanced sexually selective pressures. We wonder whether the cooler temperatures and higher precipitation of tropical environments influences the effects of sexual selection and reproductive isolation mechanisms on CHC modification because these seemingly species-rich environments require species boundaries. Very few arid species have been tested for their ability to mate in the dark (Fig. 2, Table 1). Based on the lack of visual communication that has been observed in arid species from field studies (O'Grady and Markow, 2012), perhaps non-visual communication is more efficient as arid *Drosophila* may rely on temperature-defined timing in activity in order to escape the extreme conditions. Further comparative investigations of CHC profiles and disentangling the phylogenetic and ecological relevance of CHCs and pheromones across many *Drosophila* species could offer insight into such hypotheses. Arid species show the most differences in the size of their CHC chains (Table 1, Fig. 3) compared with tropical and temperate species. This, in addition to their conserved desiccation tolerance (Kellermann et al., 2012a), suggests genetic adaptations acting on CHC metabolism may have occurred that enabled these species to colonize arid habitats.

Throughout this Review, we have made broad generalizations across species through 'climatic categories'. These categories are based on literature annotations, and each encompasses a wide range of localities spanning the globe (see Fig. 1 and Table 1). Although modelling abiotic conditions, such as temperature and precipitation, should be regional enough to make conclusions valid, we have not considered confounds from species that could have been sampled from microclimates. For instance, arid species, such as *D. buzzatii*, have been collected from arid corridors of otherwise tropical areas of Brazil (Oliveira et al., 2011); thus pressures concerning water and precipitation will be different than in arid areas adjacent to temperate regions. This highlights the difficulties in making generalizations, even within a species, and underscores the need to record where and under what conditions the samples are acquired in any study. We are similarly aware that the adaptability of certain types (especially cosmopolitan) can generate species that do not conform to the

Table 1. Generalized distribution, dependence of light on mating, average CHC length and CHC sexual dimorphism of the species mentioned throughout this Review

Species name	Species group	Distribution	Visual signalling dependence	Average CHC length	Sexual dimorphism in CHC profile	
<i>melanogaster</i>	<i>melanogaster</i> subgroup	Cosmopolitan ¹	Class I ^{4,5}	25.31 ^{11,21,22}	Dimorphic ¹¹	
<i>simulans</i>		Cosmopolitan ¹	Class II ⁴	25.00 ^{11,22,23}	Monomorphic ¹¹	
<i>sechellia</i>		Tropical ¹		25.50 ¹¹	Dimorphic ¹¹	
<i>mauritiana</i>		Tropical ¹	Class III ⁶	23.00 ¹¹	Monomorphic ¹¹	
<i>yakuba</i>		Tropical ¹	Class I ⁵	25.00 ^{11,24,25}	Monomorphic ¹¹	
<i>santomea</i>		Tropical ¹		24.83 ²⁵	Monomorphic ¹²	
<i>teissieri</i>		Tropical ¹		23.00 ¹¹	Monomorphic ¹¹	
<i>erecta</i>		Tropical ¹		28.00 ¹¹	Dimorphic ¹¹	
<i>suzukii</i>		<i>suzukii</i> subgroup (<i>melanogaster</i> group)	Cosmopolitan ²	Class III ^{4,5}		Monomorphic ¹³
<i>takahashii</i>		<i>takahashii</i> subgroup (<i>melanogaster</i> group)	Tropical ¹	Class II ⁴		
<i>auraria</i>		<i>montium</i> subgroup (<i>melanogaster</i> group)	Tropical ¹	Class III ^{4,5}		
<i>kikkawai</i>			Tropical ¹	Class I ⁵		
<i>rufa</i>			Temperate ¹	Class II ⁴		
<i>birchii</i>			Tropical ¹		25.12 ^{26,27}	
<i>serrata</i>		Tropical ¹		26.81 ^{26,27}		
<i>elegans</i>	<i>elegans</i> subgroup (<i>melanogaster</i> group)	Tropical ¹		24.14 ²⁸		
<i>ananassae</i>	<i>ananassae</i> subgroup (<i>melanogaster</i> group)	Cosmopolitan ²	Class I ^{4,5,7}			
<i>pallidosa</i>		Tropical ¹		33 ²⁹		
<i>malerkotliana</i>		Cosmopolitan ¹	Class II ⁴			
<i>bipectinata</i>		Tropical ¹	Class II ⁵			
<i>subobscura</i>	<i>obscura</i> group	Temperate ¹	Class III ⁴	26.67 ³⁰	Dimorphic ¹⁴	
<i>pseudoobscura</i>		Temperate ¹	Class I ⁴	28.49 ³¹		
<i>persimilis</i>		Temperate ¹	Class II ⁴	25.00		
<i>willistoni</i>	<i>willistoni</i> group	Tropical ¹	Class I ⁸			
<i>nebulosa</i>		Tropical ¹	Class III ⁸			
<i>prosaltans</i>	<i>saltans</i> group	Tropical ¹	Class II ⁴			
<i>melanica</i>	<i>melanica</i> group	Temperate ¹	Class III ⁴			
<i>robusta</i>	<i>robusta</i> group	Temperate ¹	Class II ⁴			
<i>virilis</i>	<i>virilis</i> group	Temperate ¹	Class I ⁴		Dimorphic ^{15,16}	
<i>kanekoi</i>		Temperate ¹		24.67 ¹⁶	Dimorphic ^{15,16}	
<i>littoralis</i>		Temperate ¹		24.75 ¹⁶	Monomorphic ¹⁶	
<i>lummei</i>		Temperate ¹		25.38 ¹⁶	Monomorphic ¹⁶	
<i>americana</i>		Temperate ¹		21.00 ¹⁶	Dimorphic ¹⁶	
<i>novamexicana</i>		Arid ¹		21.00 ¹⁶	Dimorphic ¹⁶	
<i>borealis</i>		Temperate ¹		25.67 ¹⁶	Monomorphic ¹⁶	
<i>montana</i>		Temperate ¹		27.47 ¹⁷	Dimorphic ¹⁷	
<i>laticola</i>		Temperate ¹		26.67 ¹⁷		
<i>mulleri</i>	<i>mulleri</i> subgroup (<i>repleta</i> group)	Arid ¹	Class II ⁴			
<i>mojavensis</i>		Arid ¹		33.15 ¹⁸	Dimorphic ¹⁸	
<i>navojoa</i>		Arid ¹		33.10 ¹⁸		
<i>arizonae</i>		Arid ¹		33.42 ¹⁸		
<i>buzzatii</i>		Arid ¹		32.60 ³	Dimorphic ³	
<i>serido</i>		Arid ¹		32.38 ³	Dimorphic ³	
<i>antonietae</i>		Arid ³		29.50 ³	Dimorphic ³	
<i>gouveai</i>		Arid ³		33.67 ³	Dimorphic ³	
<i>seriema</i>		Arid ³		33.08 ³	Dimorphic ³	
<i>borborema</i>		Arid ¹		33.00 ³	Dimorphic ³	
<i>koepferae</i>	Arid ¹		32.83 ³	Dimorphic ³		
<i>mercatorum</i>	<i>mercatorum</i> subgroup (<i>repleta</i> group)	Cosmopolitan ¹	Class I ⁹			
<i>hydei</i>	<i>hydei</i> subgroup (<i>repleta</i> group)	Cosmopolitan ¹	Class I ⁴			
<i>pavani</i>	<i>mesophragmatica</i> group	Tropical ¹	Class III ⁴			
<i>gibberosa</i>	<i>annulimana</i> group	Tropical ¹	Class III ⁴			
<i>grimshawi</i>	Hawaiian picture wing clade	Tropical ¹	Class III ⁴	26.00 ¹⁹	Monomorphic ¹⁹	
<i>adiastola</i>		Tropical ¹			Dimorphic ¹⁹	
<i>immigrans</i>	<i>immigrans</i> group	Cosmopolitan ¹	Class I ⁴	28.00 ³⁰		
<i>kepuluana</i>	<i>nasuta</i> subgroup (<i>immigrans</i> group)	Tropical ¹	Class III ⁴			
<i>sulfurigaster</i>		Tropical ¹	Class III ⁴			
<i>albomicans</i>		Tropical ¹	Class III ⁴	26.89 ³³		
<i>nasuta</i>		Tropical ¹		26.89 ³³		
<i>pallidifrons</i>		Tropical ¹	Class III ⁴			
<i>kohkoa</i>		Tropical ¹	Class III ⁴			
<i>pulaua</i>		Tropical ¹	Class III ⁴			
<i>innubila</i>		Arid ¹	Class III ⁴			
<i>acutilabella</i>	<i>cardini</i> group	Tropical ¹	Class III ⁴			
<i>tripunctata</i>	<i>tripunctata</i> group	Temperate ¹	Class III ⁴			

Continued

Table 1. Continued

Species name	Species group	Distribution	Visual signalling dependence	Average CHC length	Sexual dimorphism in CHC profile
<i>phalerata</i>	quinaria group	Temperate ¹	Class III ⁴	28.00 ¹⁷	Dimorphic ²⁰
<i>subquinaria</i>		Temperate ¹	Class II ¹⁰	32.00 ²⁰	
<i>recens</i>		Temperate ¹	Class II ¹⁰	32.00 ²⁰	
<i>falleni</i>		Temperate ¹	Class II ⁴		
<i>occidentalis</i>		Temperate ¹	Class II ⁴		
<i>subpalustris</i>		Temperate ¹	Class II ⁴		
<i>guttifera</i>		Temperate ¹	Class III ⁴		
<i>palustris</i>	Arid ¹	Temperate ¹	Class III ⁴		
<i>munda</i>		Class III ⁴			
<i>funnebris</i>	funnebris group	Cosmopolitan ²	Class I ^{4,7}		

Each species was placed into one of four 'climatic categories' depending on their known collection sites. A species visual dependence for mating behaviour was (Class I) light independent, (Class II) facultative dark mating, or (Class III) dark repressed (Grossfield, 1971). The average cuticular hydrocarbon (CHC) length was estimated by averaging the length of the hydrocarbon backbone (in carbons) for all CHC compounds identified in the literature for each respective species, and the CHC dimorphism indicates whether males and females express their CHC profiles differentially, based on qualitative or quantitative differences in their CHC profile. The shaded cells indicate an absence of known data and allow the visualization of gaps in behavioural comparisons across *Drosophila* species.

¹Markow and O'Grady 2006; ²Markow and O'Grady, 2008; ³Oliveira et al., 2011; ⁴Grossfield, 1971; ⁵Hardeland, 1972; ⁶Robertson, 1983; ⁷Hardeland, 1971; ⁸Gleason et al., 2012; ⁹Ikeda, 1976; ¹⁰Giglio and Dyer, 2013; ¹¹Cobb and Jallon, 1990; ¹²Mas and Jallon, 2005; ¹³Dekker et al., 2015; ¹⁴Toolson and Kuper-Simbron, 1989; ¹⁵Jackson and Bartelt, 1986; ¹⁶Bartelt et al., 1986; ¹⁷Jennings et al., 2014; ¹⁸Etges and Jackson, 2001; ¹⁹Alves et al., 2010; ²⁰Curtis et al., 2013; ²¹Farine et al., 2012; ²²Rouault et al., 2004; ²³Bontonou et al., 2012; ²⁴Denis et al., 2015; ²⁵Mas and Jallon, 2005; ²⁶Howard et al., 2003; ²⁷Frentiu and Chenoweth, 2010; ²⁸Ishii et al., 2001; ²⁹Ferveur, 2005; ³⁰Hedlund et al., 1996; ³¹Blomquist et al., 1985; ³²Noor and Coyne, 1996; ³³Kim et al., 2013.

general conclusions and 'categorizations' of tropical, temperate or arid. Rather, these categories are fluid based on the collection sites, and how long these species are bred under constant conditions within the laboratory. We see the identification and study of species within these categories as a way of understanding how adaptations to the environment can have far-reaching implications, and intra-specific variation across habitats is an incredibly exciting area of study.

The currently accepted *Drosophila* phylogeny is quite comprehensive, with over 180 species (van der Linde et al., 2010). Future comparative studies need to sample species based on phylogenetic relationships, much like Kellermann and colleagues (Kellermann et al., 2012a,b). Such studies, whether they are behavioural, physiological or genetic, may dispel generalizations we have made, such as all *repleta* species being arid in distribution with high stress resistance and long CHCs. In fact, *D. ellisoni*, *D. pavani* and *D. gibberosa* are such exceptions as they are distributed in tropical regions, yet are not distantly related to *D. mojavensis*. In fact, *D. ellisoni* has low desiccation and cold tolerance, unlike its close relatives (Kellermann et al., 2012a). Does this species behave similarly to *D. mojavensis* at the social level? Or does it behave more like other tropical species? Does it mate in the dark? Does it have a similar hydrocarbon profile? Such questions can easily be answered and may offer valuable insight into how visual, chemical and other sensory modalities in *Drosophila* evolve based on environmental selective pressures.

Untangling these possibilities to understand the causes of variation between the species will ultimately rely on identifying the gene pathways and mechanisms responsible for the adaptation to the ecological and social pressures. Although we are at the stage where identifying gene pathways associated with behavioural evolution is more difficult in species other than *D. melanogaster*, studies have been conducted that were dedicated to untangling the phenotypic aspects of social communication during courtship. Such studies involved physically blinding flies (Gleason et al., 2012; Grossfield, 1971; Giglio and Dyer, 2013; Wallace and Dobzhansky, 1946), removing antennae to crudely ablate olfaction (Gleason et al., 2012; Giglio and Dyer, 2013) and removing wings to remove auditory and visual cues (Fuyama, 1979; Giglio and Dyer, 2013;

Gleason et al., 2012; Grossfield, 1968). Recent publications have conducted all of the above sensory disruptions on *D. willistoni* and *D. nebulosa* (Gleason et al., 2012) and on *D. subquinaria* and *D. recens* (Giglio and Dyer, 2013) to untangle the influence of each sensory modality on courtship, and each study used two species that are closely related. More studies similar to these are desperately needed. This may lead to the identification of evolutionary mechanisms at play in the adaptation of *Drosophila* behaviour to the social group and other features of their environment.

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Competing interests

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