Austral Entomology

Austral Entomology (2022) **61**, 370–377

Climatic and biotic influences on the distributions of *Calliphora augur* and *Calliphora dubia* (Diptera: Calliphoridae)

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Abstract *Calliphora augur* (Fabricius) and *Calliphora dubia* (Macquart) are two widespread and endemic Australian blowflies of applied importance. In order to better understand the biology of these species, this study used historical, field and laboratory data to determine and interpret their distributions. Locality records from insect collections and the literature were used to determine the known distributions of each species. The resultant maps were partially verified with field trapping data for each species. *Calliphora augur* was found to dominate in the east of Australia, while *C. dubia* dominates in the west. Comparative laboratory and field experiments were also used to derive parameters defining the distributions of these two closely related species, particularly in relation to temperature and moisture. *Calliphora dubia* had a greater tolerance for dry stress and high temperatures than *C. augur*, although maggots of both species displayed similar developmental temperature preferences. No difference was seen between species in the impact of low temperatures on the development of maggots through to the pupal stage. The greater ability of *C. dubia* to tolerate adverse environmental conditions is most likely linked to the shared evolutionary history of both species, in which increased aridity in central Australia is proposed to have triggered speciation by separating ancestral populations: *C. dubia* evolved in the hot, dry conditions in the west of Australia, while *C. augur* evolved in the cooler, moist environment of the east. Improved understanding of the influences on the distributions of these common Australian blowflies will assist in the further study of their application to agriculture and forensic science.

Key words Australia, blowfly, dry stress, moisture, temperature, threshold, tolerance.

INTRODUCTION

Documenting the known and potential distributions of flies is crucial to understanding their quarantine risk and role in agriculture, pest management, and medical, forensic and veterinary science. *Calliphora augur* (Fabricius) and *Calliphora dubia* (Macquart) are two notable Australian blowfly species of agricultural importance as agents of myiasis in sheep and as potential indicators of time since death in forensic entomology (Norris 1959; Levot 2003).

The two species are very similar morphologically: *C. dubia* was originally only recognised as different from *C. augur* based on the colouration of the abdomen (Hardy 1932). *Calliphora augur* has a dorsally greenish-blue abdomen with yellowish dust on the fifth tergite (dorsal abdominal plate), while the abdomen of *C. dubia* is rich blue or purplish with vivid white dust on the fifth tergite. The species also differ in the dimensions of the eyes and the frons (Hardy 1932). They have also been regarded as subspecies (*C. augur augur* and *C. augur dubia*) (Kurahashi 1971) because of their extensive morphological similarity as both juveniles and adults (Wallman 2001a, 2001b). Furthermore, the low levels of discrimination between the two on the basis of mitochondrial DNA (mtDNA) suggest a very recent divergence; the species have yet to be recovered as reciprocally monophyletic in mitochondrial phylogenetic analyses (Wallman & Donnellan 2001; Harvey *et al*. 2003).

Calliphora augur and *C. dubia* are ovoviviparous, laying batches of about 50 larvae sheathed in the chorion (the eggshell), which hatch almost instantaneously following release. The larvae begin feeding soon after deposition (Mackerras 1933; Norris 1959; Callinan 1980; Cook & Dadour 2011).

Both *C. augur* and *C. dubia* are endemic to Australia. *Calliphora augur* is known to exist throughout south-eastern Australia, whereas *C. dubia* is found throughout south-western and central-southern Australia. Their distributions broadly overlap between these two regions, and they co-occur around eastern South Australia (SA), Kangaroo Island and western New South Wales (NSW) and western Victoria (Wallman & Adams 1997). Like many calliphorids, their distributions also vary seasonally. *Calliphora augur* is generally absent over winter, with a population maximum during early to mid-summer, and a relatively high abundance sustained throughout autumn (Norris 1959; Archer & Elgar 2003). In Adelaide, *C. dubia* substitutes for *C. augur*, but its seasonal occurrence is

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doi: 10.1111/aen.12617

comparable with *C. augur*, except that there is a shorter absence in winter (Norris 1959). On the tableland of south-western Australia, *C. dubia* may be active throughout the year but is most abundant throughout autumn and spring (Norris 1959). Understanding seasonal changes in these populations is crucial for gaining an overview of the distributions of these flies, and thus, exploring the factors impacting on this seasonality should be a priority.

This study investigated the biogeography of *C. augur* and *C. dubia* as a means of better understanding the ecology and evolution of these Australian blowflies. Locality data were analysed and partially verified by field trapping. Experiments were also conducted to determine climatic variables that might influence the species' distributions.

MATERIAL AND METHODS

Determining current distributions

A database of known localities for *C. augur* and *C. dubia* was constructed using preserved specimens (collections from the Australian National Insect Collection (Canberra, Australia)), as well as the institutional collections of Wallman (University of Wollongong (UOW)) and Melanie S. Archer (Victorian Institute of Forensic Medicine) and locality data from the literature (Palmer 1975; O'Flynn 1976; Monzu 1977; O'Flynn 1980; McQuillan *et al*. 1983; Morris 1993). Latitude and longitude coordinates for each location were entered into the geographic mapping software ArcGIS 9 (ESRI Inc., USA) to create the first detailed maps of the known distributions of the two species.

Confirming current distributions

The resultant maps, combined with other information from the literature, suggested a zone of sympatry between *C. augur* and *C. dubia* from western New South Wales and Victoria to the eastern edge of South Australia. A sampling transect from east to west was carried out, during February 2007, to supplement the existing data and refine the supposed area of sympatry. The sampling transect began at Berry Beach (34.79°S, 150.76°E), on the east coast of New South Wales, and ended in Adelaide (34.92°S, 138.41°E), on the south coast of South Australia. Both species are known to occur at each location, but *C. augur* is more dominant at Berry Beach, and *C. dubia* dominates in Adelaide (pers. obs.). Trapping of flies was undertaken approximately every 300 km between these two sites at Yass, NSW (34.84°S, 148.92°E), Narrandera, NSW (34.77°S, 146.57°E), Balranald, NSW (34.13°S, 143.52°E) and Pinnaroo, SA (35.25°S, 140.90°E). Additional trapping was done at Wagga Wagga, NSW (35.10°S, 147.37°E) after an initial analysis of trap yields suggested that the most distinct cross-over between the distributions of the two species lay somewhere between Yass and Narrandera.

Sampling at each site was done using three 'West Australian' traps baited with approximately 1000 g of chopped beef liver, 330 g of kangaroo mince and 500 mL of sodium sulphide solution (2% Na_2S in water), a mixture used with success in other blowfly population studies (Kavazos & Wallman 2012). The base of the supporting post of each trap was treated with petroleum jelly and a granulated insecticide to prevent ant invasion from the ground.

Traps were placed approximately 50 m apart in lightly vegetated areas with dappled shade, on the outskirts of each town. Traps were set up at one of three time points: 09:00, 12:00 or 17:00, subject to the time of arrival at each location. The bait was left for 24 h and stirred at the remaining two time points to prevent a film forming on the surface of the bait. This film seemed to reduce bait odour and hence attractiveness to flies (pers. obs.).

At the end of the 24 h period, each trap was collected and placed in a heavy-duty plastic bag, together with a sponge soaked in ethyl acetate. The bag was sealed and left for 5–10 min until all flies were dead. The dead flies were removed and preserved in 95% ethanol. Specimens of *C. augur* and *C. dubia* in each trap were identified using a dissecting microscope.

The total number of flies collected at each site was recorded to give the proportions of *C. augur* and *C. dubia* relative to the total trap catch. If no *C. augur* or *C. dubia* were present in a trap (0 flies), the 0 was converted to 0.1. This eliminated invalid responses in the statistical software when values of 0 were encountered, without biasing the results.

Maximum and minimum temperatures, along with average rainfall, and 9:00 and 15:00 relative humidity values, were obtained from the Australian Bureau of Meteorology for each location on the days on which trapping was carried out. A logistic regression was applied, using the statistical package SAS 9.1 (SAS Institute, USA), to relate the adjusted proportions of *C. augur* and *C. dubia* to the effect of longitude and associated climatic data.

Temperature tolerance

In order to study the ability of these flies to deal with temperature extremes, experiments were conducted on the development of each species at 35°C, 37°C and 40°C. While it is unlikely that either species would be constantly exposed to such temperatures, they reflect the upper annual average daily maximums encountered in Australia (Bureau of Meteorology 30 year climatology (1976–2005), Australia).

These and subsequent temperature experiments used established fly colonies at UOW. These cultures were established from individuals from Wollongong, NSW (34°24′S, 150°52′E) for *C. augur* and from Balranald, NSW (34°13′S, 143°52′E) for *C. dubia*. These two localities were chosen as the source of the cultures as they represent locations typical of the main distributions of each species but removed from the zone of cross-over. Hence, the risk of hybridisation or misidentification was minimised.

Cultures were maintained in 30 × 50 × 25 cm plastic cages with a fly screen lid. They were provided with granulated raw sugar and water *ad libitum*. A small portion of sheep's liver in a plastic weigh boat was provided for ovary maturation and as a substrate for larviposition. Colonies were checked daily for maggot production. Liver and newly larviposited maggots were

removed and placed into a 13 × 19 × 7 cm plastic rearing container with a fine mesh top. The bottom of the container was covered with wheaten chaff as a pupation material. Extra liver was given to the maggots when necessary to ensure they did not become food deprived. Upon pupation, the rearing container, along with pupae, was relocated to a fresh cage, and the lid removed to permit the free movement of newly emerged adults. The colony was kept in a temperature-controlled room maintained at 25 ± 3°C and a 12:12 light : dark regime, with a 15 min transition period of low light between light and dark.

Maggots from the cultures were collected within 1 h of laying and placed on 50 g portions of liver. Three replicates of 20 maggots were used per temperature for each species. All experiments were logged using a Thermotag™ temperature logger (Thermodata, Australia), which revealed an error margin of ±2°C within the temperature cabinets. We measured (1) maggot survivorship, monitored every 2 to 3 days with individuals removed as they died, (2) pupation success (confirmed by shining a bright light through the wall of the puparium to observe the pupa within, with dissection as necessary) and (3) adult eclosion. Survivorship of the two species at each temperature was compared using Student's *t*-tests.

Temperature preferences

In order to examine the preferred temperature range of each larval instar of *C. augur* and *C. dubia* during their development, a temperature gradient apparatus was used to provide a range of temperatures between approximately 20°C and 45°C as per Johnson *et al*. (2014). One end of the tray was attached to a heating element that maintained a constant temperature of 60°C, while the other end was cooled using an ice bath.

Kangaroo mince was mixed with soil water crystals at a ratio of 3:2 and then spread evenly across the tray. The heating apparatus was switched on and left to heat for 2 h. After 2 h, maggots of the desired instar were obtained from the UOW stock cultures and placed on the meat in the gradient apparatus. A total of 30 maggots were used, with five individuals placed every 100 mm along the length of the gradient. Maggots were left for 2 h, after which their positions were recorded, along with the temperature across the gradient. This procedure was repeated for each instar. For the third-instar larvae ready to pupate, the mince in the gradient apparatus was replaced with wheaten chaff as a pupation medium and larvae left for 2 days to allow completion of pupation.

Effect of dry stress

One hundred and twenty pupae from the UOW stock cultures were divided evenly into four plastic cages (30 pupae per cage) with screen tops roughly 300 × 500 × 250 mm in size. These cages were then placed inside a temperature-controlled incubator (Thermoline, Australia) at the experimental temperature (±1°C), and puparia left in the presence of water and sugar for adults when they eclosed, and checked daily. No source of protein was provided so as to prevent the newly emerged flies reproducing. This setup was replicated for each species and with experimental temperatures of 15°C, 20°C and 25°C. The three temperatures used for these experiments were chosen based on the optimal temperature ranges for *C. dubia* and *C. augur* determined in the above experiments.

Seven days after the first sign of eclosion, the puparia from which adults had not eclosed were removed. After a further 7 days, water (but not sugar) was removed concurrently from three of the four cages for each species and temperature treatment. Thus, water was removed 2 weeks after the first flies had eclosed, and time of water removal represented day zero for all survivorship curves. The flies in the fourth cage that retained access to water acted as controls.

Following the removal of water, all cages (including the controls) were checked daily for mortality until all flies were dead. Mortality prior to the onset of the treatments was minimal and excluded from analyses.

Survivorship curves were created by calculating survival fractions using the product limit (Kaplan–Meier) method. These were then compared using a log-rank (Mantel–Cox) approach. Standard errors were based on the uncertainty of fractional survival using Greenwood's formula. All analyses were carried out using GraphPad Prism™ Software, version 7.0 (USA), with significance set to $\alpha = 0.05$.

RESULTS

Determining current distributions

The collection and literature data collectively provided records for the two species across the majority of Australia south of about 20°S. *Calliphora augur* is distributed throughout the east, with *C. dubia* showing an even broader range across the west and central-southern areas of Australia. Kangaroo Island, SA (~137°E) denotes the approximate westerly limit of *C. augur*, although *C. dubia* has been recorded as far east as Berry Beach, NSW (~150°E) (Fig. 1).

Confirming current distributions

The transect across south-eastern Australia showed a clear decline in the number of *C. augur* caught as trapping moved further west, corresponding with an increase in *C. dubia* numbers (Fig. 2).

The logistic regression found the proportion of *C. augur* trapped to be significantly positively correlated with longitude ($P < 0.0001$), maximum and minimum temperature ($P < 0.0001$) and the relative humidity at 15:00 ($P < 0.0001$). The relative humidity at 9:00 did not have a significant correlation with the proportion of *C. augur* caught ($P = 0.665$), and the number of *C. dubia* present had a significantly negative correlation with *C. augur* proportions ($P = 0.0317$).

Similarly, the proportion of *C. dubia* trapped was found to be significantly correlated with longitude ($P < 0.0001$), maximum and minimum temperature ($P = 0.0003$ and $P < 0.0001$, respectively) and the relative humidity at 15:00 ($P < 0.0001$). However, in contrast to *C. augur*, these factors all had a negative correlation, while relative humidity at 9:00 and the number of *C. augur* present both had a significantly positive correlation on *C. dubia* proportions ($P < 0.0001$). Rainfall was not a

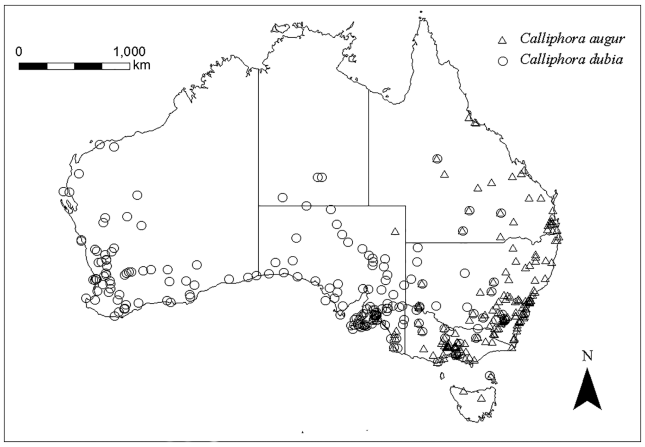

Fig. 1. Distribution of *Calliphora augur* and *Calliphora dubia* as indicated from pinned institutional specimens and the literature. Background data: Geoscience Australia – Global Map Australia 1M 2001.

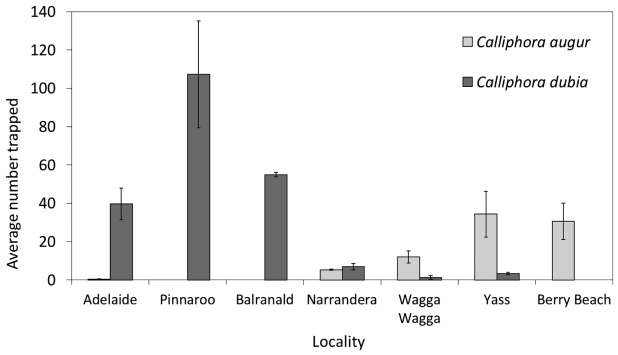

Fig. 2. Average number of *Calliphora augur* and *Calliphora dubia* caught in traps at each location, west to east (error bars show ±SE).

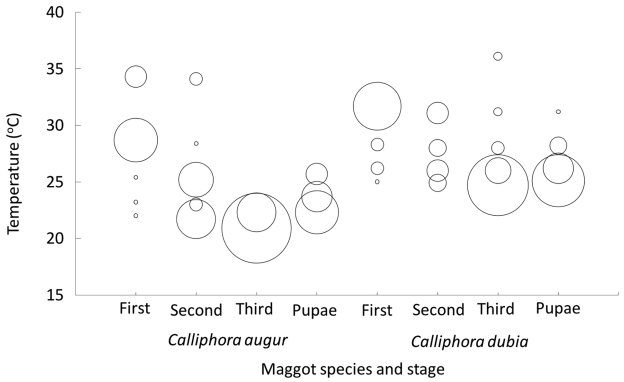

Fig. 3. Distributions of maggots of *Calliphora augur* and *Calliphora dubia* along the temperature gradient. Circles represent groups of maggots at a particular temperature. The temperature is indicated by the centre of the circle, while the radius is proportional to the number of maggots present.

relevant factor as no rain fell at any of the localities during the trapping periods.

Temperature tolerance

Maggots of both *C. augur* and *C. dubia* were unable to survive at either 40°C or 37°C. All maggots died after 2 days at 40°C, while at 37°C significantly more *C. dubia* larvae than *C. augur* reached the wandering stage before dying ($t_{4,0.5} = 3.046$, $P < 0.05$). At 35°C significantly more *C. dubia* larvae pupated than did those of *C. augur* ($t_{4,0.5} = 2.792$, $P < 0.05$). However, pupation success was reduced for both species (60% remaining for *C. dubia* and 40% for *C. augur*), and pupae at dissection were mostly found to be dead.

Temperature preferences

The temperature gradient had a mean range from 21°C to 44°C (Fig. 3). A between-species comparison found that *C. augur* maggots selected significantly lower temperatures than those of *C. dubia* ($F_{1, 175} = 23.20$, $P < 0.0001$). The mean (+SD) temperatures chosen by *C. augur* maggots within the first, second and third instars, and for pupation, were 29.4 (+3.7), 25.6 (+5.5), 21.4 (+0.7) and 23.5 (+1.4)°C, respectively. For *C. dubia*, the mean (+SD) temperatures chosen by first, second and third instars, and for pupation, were 29.8 (+2.5), 27.6 (+0.5), 26.7 (+3.3) and 26.2 (+1.6)°C, respectively. A significant difference in the temperatures selected by maggots was seen among instars ($F_{3, 86} = 20.46$, $P < 0.0001$ and $F_{3, 83} = 7.93$, $P < 0.0001$) for both *C. augur* and *C. dubia*, respectively. Specifically, first-instar maggots of both species selected higher temperatures than did the third-instar larvae for either feeding or pupation (Fig. 3).

Effect of dry stress

25°C treatment

No significant differences between the survivorship curves of *C. dubia* and *C. augur* adults were observed at 25°C when flies had free access to water throughout the experiment ($\chi^2_1 = 3.15$, $P = 0.08$) (Fig. 4a). Without water, *C. dubia* survived

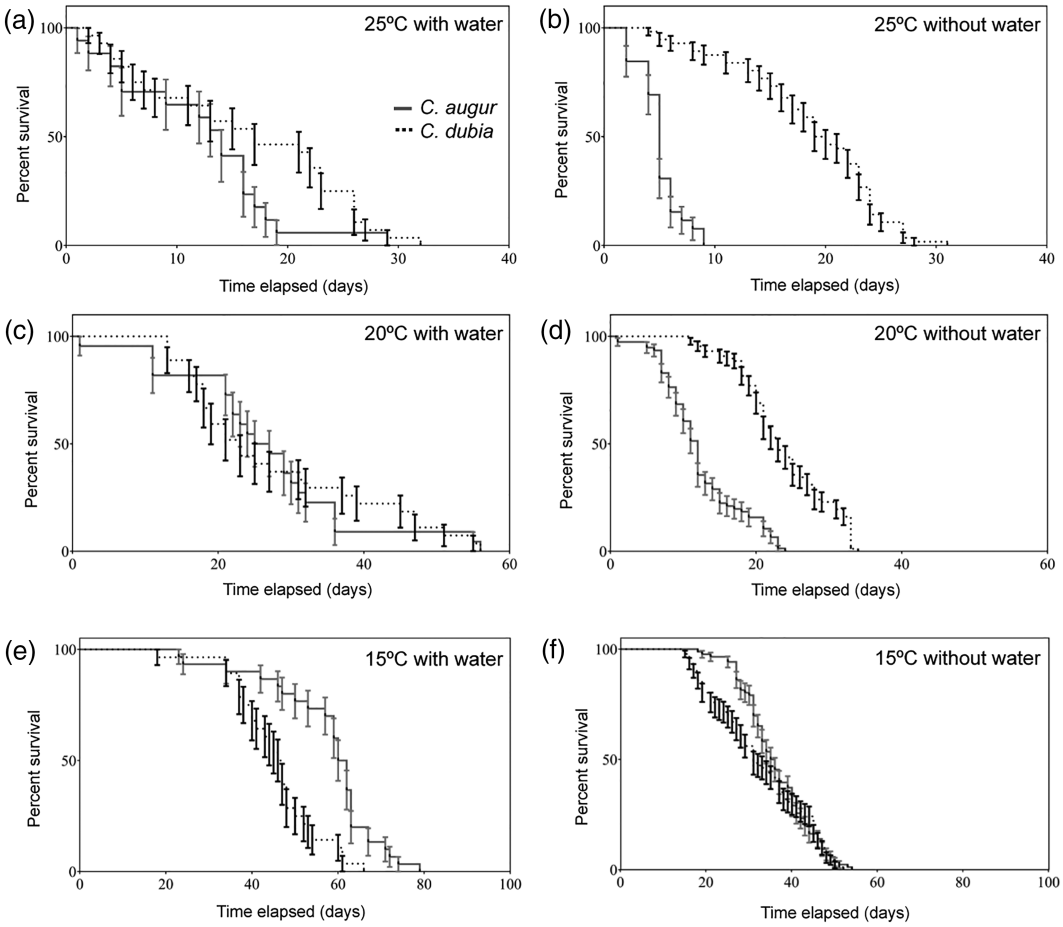

Fig. 4. Survivorship curves for *Calliphora dubia* (black broken line) and *Calliphora augur* (grey solid line) in days at (a) 25°C when provided with water, (b) 25°C when deprived of water, (c) 20°C when provided with water, (d) 20°C when deprived of water, (e) 15°C when provided with water and (f) 15°C when deprived of water. Onset of experiments and daily recording of mortality began with mature flies approximately 14 days old. Survivorship curves are derived from percentage survival, and error bars represent standard errors based on Greenwood's formula.

significantly longer than *C. augur* ($\chi^2_1 = 82.59$, $P < 0.0001$), with *C. dubia* surviving for a median of 19.5 days, whereas the median survival of *C. augur* was only 5 days after removal of water (Fig. 4b).

20°C treatment

As in the 25°C experiments, no significant differences in survivorship were seen between the species when water was provided at 20°C ($\chi^2_1 = 0.01$, $P = 0.93$) (Fig. 4c). After water was removed, *C. dubia* survived a median of 23 days, whereas *C. augur* survived a median of 12 days (Fig. 4d). The survivorship curve for *C. dubia* was significantly different from that of *C. augur* ($\chi^2_1 = 98.15$, $P < 0001$).

15°C treatment

Calliphora augur lived significantly longer with water at 15°C than *C. dubia* ($\chi^2_1 = 20.20$, $P < 0.0001$) (Fig. 4e); however, no significant differences were seen in survivorship when water was removed ($\chi^2_1 = 1.542$, $P = 0.2143$), with median survivals of 31 and 35 days for *C. dubia* and *C. augur*, respectively (Fig. 4f).

DISCUSSION

Distribution

The distributions of *C. augur and C. dubia* derived from pinned specimens and the literature are similar to those previously identified by both Monzu (1977) and Morris (1985), although the ranges of both species, particularly *C. augur*, have been extended by the current work. This is the first time that all relevant distribution data have been combined for these species.

The results clearly demonstrate that *C. augur* dominates in eastern Australia, while *C. dubia* is distributed throughout inland and western Australia. This distinction was further confirmed by the transect data. The logistic regression revealed that the number of trapped *C. augur* increased as traps were located further east. The inverse was seen for *C. dubia*, supporting the eastern *C. augur* and western *C. dubia* distribution seen in the current distribution map. This study has significantly refined knowledge of the distributions of these species by compiling disparate historical records into an easily accessible map and confirming the historical data with a new approach.

It is unknown from these data whether one species competitively dominates the other throughout the sympatric zone of their distributions. However, the presence of *C. dubia* had a significant negative effect on the probability of catching *C. augur*, which may suggest a level of competition, with *C. dubia* reducing the numbers of *C. augur*. Conversely, the presence of *C. augur* had a positive effect on the proportion of *C. dubia*, also suggesting that *C. dubia* holds a competitive advantage, with numbers not being depressed by the presence of *C. augur*. This pattern may also be a product of the relative environmental favourability of the location for each species, although these two hypotheses are potentially interactive rather than mutually exclusive. Further study of these species, which are thought to have arisen by allopatry (Wallman *et al*. 2005), is needed to fully appreciate the interactions that occur in their zone of sympatry. Such interactions in other taxa frequently involve high levels of competition when populations re-establish contact after allopatric speciation events (Bush 1975).

There was a greater proportion of *C. augur* in conjunction with increased minimum and maximum temperatures, as well as with increased relative humidity at 15:00, while the proportion of *C. dubia* decreased. This increase of *C. augur* and decrease in *C. dubia* with increasing temperature is surprising, especially given the correlation of the distribution of *C. augur* with cooler climates and that of *C. dubia* with warmer climates. It may be that annual temperatures have a greater effect on trap numbers than daily temperatures for a given trapping period. Alternatively, temperature may be a minor factor in the control of the distributions of these species. *Calliphora augur* thrives in moist areas, where rainfall and humidity are higher, while *C. dubia* dominates in drier climates, where rainfall and humidity are lower. This association between moisture and distribution is not surprising given their presumed evolutionary origins, with *C. dubia* evolving to cope with the drier conditions in the west of the Australian continent, while *C. augur* evolved in favour of the more mesic environment of the easterly regions, especially those east of the Great Dividing Range (Wallman *et al*. 2005).

Davidson (1933) identified the risks of comparing catches of blowflies from different localities due to the number of variables that can affect trap numbers. The current study has minimised these variables by eliminated trap bias by using traps of identical design. Furthermore, all bait was kept refrigerated until 12 h prior to setting the trap and the same type and volume of bait was used for each trap, ensuring the bait was equally attractive at all sites. While weather conditions could not be controlled, weather data were accessed from the Australian Bureau of Meteorology, and climatic conditions were factored into the analysis of trap catches. Future work should repeat the trapping effort along the same transect in order to determine whether similar results are achieved under different weather conditions and across different years.

Temperature and moisture

Although neither species was able to survive successfully at the high temperatures in the temperature tolerance experiment, *C. dubia* displayed a much greater ability to withstand these extremes than *C. augur*. This reflects the fact that the typical range of annual average daily maximums in south-western and south-central Australia, where *C. dubia* is most commonly encountered, is much higher than in south-eastern Australia where *C. augur* is mostly found (27–33°C vs. 18–27°C) (Bureau of Meteorology 30 year climatology (1976–2005), Australia).

The differences seen in the ability to survive at high temperatures are also reflected in the position of larvae of the two species along the temperature gradient. *Calliphora augur* was found to prefer significantly lower temperatures of low to mid-twenties compared to *C. dubia* at temperatures in the high twenties. *Calliphora augur* larvae also displayed a greater tendency to congregate than *C. dubia* maggots.

The fact that no significant differences in survivorship were seen between the two species when water was available at 20°C and 25°C indicates that temperature alone might not account for differences in survivability. However, the inverse results seen at 15°C, where *C. augur* survived better than *C. dubia*, show that temperature and moisture availability are not mutually exclusive factors. Although both species survived equally well with water under laboratory conditions, the secondary effects of temperature on development, reproduction and ability to effectively compete in the wild should not be overlooked. Indeed, in addition to abiotic factors such as temperature and precipitation, inter-specific competition is likely to affect the range and distribution of *C. dubia* and *C. augur* in Australia.

The absence of drinking water resulted in significant differences in mortality between *C. dubia* and *C. augur*, particularly at 25°C. As expected, based on known distribution, *C. dubia* demonstrated superior ability to survive without water. Because the differences in survivability were greatest at 25°C compared to 20°C and 15°C, it is reasonable to conclude that these adaptations are at least partly temperature sensitive. Moreover, despite rearing under identical conditions for multiple generations, differences in response to dry environments persist, suggesting a genetic basis for these adaptations.

Resistance to desiccation in insects can arise either by tolerating a larger percentage of body water loss, by increasing body water storage or by reducing the rate at which water is lost to the environment (Gibbs & Matzkin 2001). Of these three adaptations, tolerance to water loss in non-diapausing adults appears to vary the least between species (Addo-Bediako *et al*. 2001). Experiments on selectively bred *Drosophila* (Gibbs *et al*. 1997; Archer *et al*. 2007), as well as comparative examinations between closely related species (Gibbs & Matzkin 2001; Gray & Bradley 2005), have not found water content at death to vary between populations adapted to mesic vs. xeric environments. Gray and Bradley (2005) postulated that early adaptation to a terrestrial environment may have already pushed dehydration tolerance to its physiological limits in some dipterans, eliminating genetic variability for this trait.

In contrast, increases in stored body water have been consistently demonstrated to be important adaptive responses to aridity. Bulk water storage in the form of increased haemolymph volume has been correlated with desiccation resistance (Gibbs *et al*. 1997), and haemolymph water reserves can be augmented

by high glycogen content (Folk *et al*. 2001; Archer *et al*. 2007). Glycogen can bind up to five times its weight in water and can therefore also increase the source of 'bound' water in insects that use this substrate for energy (Schmidt-Nielsen 1997). Whether mass-specific haemolymph volume or glycogen content varies between *C. dubia* and *C. augur* has not yet been examined.

By far the greatest contribution to total water loss is by transpiration through the cuticle (Gibbs 1998) and may explain the differences in desiccation tolerance seen between *C. dubia* and *C. augur*. The outermost cuticle layer, or epicuticle, is the destination of waxes that are secreted from epidermal cells and transported through pores traversing the rest of the cuticle (Blomquist *et al*. 1987). Of these waxes, the cuticular hydrocarbons (CHCs) are believed to be the principal barrier to water loss, and variability in their proportion and composition in response to environmental cues plays a large role in the water-proofing properties of the cuticle (Gibbs 1998; Nelson & Lee 2004). Insects that live in arid environments tend to express higher concentrations of long-chained (C24–C31) alkanes and monomethylalkanes than those from humid climates, which tend to express higher concentrations of shorter-chained (C19–C23) alkanes and alkenes (Chung & Carroll 2015; Menzel *et al*. 2017; Butterworth *et al*. 2020). The different properties of these CHCs result in different temperature-dependant 'transition phases' in cuticle permeability. Due to the lability of surface lipid make-up and the high contribution of cuticular water loss to desiccation, the quantity and composition of CHCs is likely a key explanation for desiccation resistance differences observed between *C. dubia* and *C. augur*. This also accounts for the temperature-sensitive nature of these differences. It is well known that CHCs vary drastically between blowfly species, particularly between closely related species that inhabit different environments (Butterworth *et al*. 2020). Further investigation of the CHCs of *C. augur* and *C. dubia* will likely provide key functional insights into these differences in their desiccation tolerance.

Evolutionary history

The approach used in this study investigated the biogeography of *C. augur* and *C. dubia* to better understand their ecology and evolution, and it also has the potential to be applied to Australian blowflies more broadly. Wallman *et al*. (2005) suggested that the relatively recent divergence of these taxa was likely due to the development of Australia's arid interior during the Pliocene and Pleistocene. This change could have resulted in the division of the ancestral population, creating the current south-eastern vs. south-western populations of *C. augur* and *C. dubia*, respectively. *Calliphora dubia* evolved to endure drier conditions in the west while *C. augur* remained adapted to the more mesic eastern environment. Not only is this hypothesis supported by the distribution data presented here but also by the experimental findings that support the greater ability of *C. dubia* to survive under heat and dry stress.

Similar recent species divergence is thought to have occurred between other species-pairs of blowflies such as *Calliphora albifrontalis* Malloch and *Calliphora stygia* (Fabricius), and *Calliphora hilli* Patton and *Calliphora varifrons* Malloch (Wallman *et al*. 2005) that also show east–west separations (Norris 1959). It is possible that the western representatives of these pairs (*albifrontalis* and *varifrons*) display equivalent abilities for tolerating dry stress compared with their eastern counterparts.

CONCLUDING REMARKS

Blowflies are common Australian insects essential to ecosystem function, but their biological aspects have not been widely studied. A critical omission is their known and potential distributions. Given the modifications to insect populations induced by climate change, we need a sound understanding of the climatic and biotic influences on the distributions of such important flies. Further study of the biological characteristics of these and other species should enable us to draw further conclusions about the evolution of Australian blowflies, while also enhancing our understanding of their ecological and applied significance.

ACKNOWLEDGEMENTS

We thank the Australian Federal Police (Canberra, Australia) and the University of Wollongong for their financial support of this study. We are also grateful to the CSIRO (Canberra, Australia) for access to the Australian National Insect Collection; Melanie Archer (Victorian Institute of Forensic Medicine, Melbourne, Australia) for locality data and miscellaneous other contributions; Leigh Nelson for assisting in the field; and Justin Lathlean, Bryan Lessard, Elizabeth Cortie and Jack Tucker for help with sorting and counting trap catches. Nathan Butterworth provided helpful comments on the manuscript.

Open access publishing facilitated by University of Wollongong, as part of the Wiley - University of Wollongong agreement via the Council of Australian University Librarians.

REFERENCES

Addo-Bediako A, Chown SL & Gaston KJ. 2001. Revisiting water loss in insects: a large scale view. *Journal of Insect Physiology* **47**, 1377–1388.

Archer MA, Bradley TJ, Mueller LD & Rose MR. 2007. Using experimental evolution to study the physiological mechanisms of desiccation resistance in *Drosophila melanogaster*. *Physiological and Biochemical Zoology* **80**, 386–398.

Archer MS & Elgar MA. 2003. Yearly activity patterns in southern Victoria (Australia) of seasonally active carrion insects. *Forensic Science International* **132**, 173–176.

Blomquist GJ, Nelson DR & De Renobales M. 1987. Chemistry, Biochemistry and Physiology of insect cuticular lipids. *Archives of Insect Biochemistry and Physiology* **6**, 227–265.

Bush GL. 1975. Modes of animal speciation. *Annual Review of Ecology and Systematics* **6**, 339–364.

Butterworth NJ, Wallman JF, Drijfhout FP, Johnston NP, Keller PA & Byrne PG. 2020. The evolution of sexually dimorphic cuticular hydrocarbons in blowflies (Diptera: Calliphoridae). *Journal of Evolutionary Biology* **33**, 1468–1486.

Callinan APL. 1980. Aspects of the ecology of *Calliphora augur* (Fabricius) (Diptera: Calliphoridae), a native Australian blowfly. *Australian Journal of Zoology* **28**, 679–684.

Chung H & Carroll SB. 2015. Wax, sex and the origin of species: dual roles of insect cuticular hydrocarbons in adaptation and mating. *BioEssays* **37**, 822–830.

Cook DF & Dadour IR. 2011. Larviposition in the ovoviviparous blowfly *Calliphora dubia*. *Medical and Veterinary Entomology* **25**, 53–57.

Davidson J. 1933. The species of blowflies in the Adelaide district of South Australia and their seasonal occurrence. *Journal of Agriculture of South Australia* **36**, 1148–1153.

Folk D, Han C & Bradley T. 2001. Water acquisition and partitioning in Drosophila melanogaster: Effects of selection for desiccation-resistance. *The Journal of Experimental Biology* **204**, 3323–3331.

Gibbs AG. 1998. Water-proofing properties of cuticular lipids. *American Zoologist* **38**, 471–482.

Gibbs AG, Chippindale AK & Rose MR. 1997. Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *The Journal of Experimental Biology* **200**, 1821–1832.

Gibbs AG & Matzkin LM. 2001. Evolution of water balance in the genus *Drosophila*. *The Journal of Experimental Biology* **204**, 2331–2338.

Gray EM & Bradley TJ. 2005. Physiology of desiccation resistance in *Anopheles gambiae* and *Anopheles arabiensis*. *The American Journal of Tropical Medicine and Hygiene* **73**, 553–559.

Hardy GH. 1932. Some Australian species of *Calliphora* (subgenera *Neopollenia* and *Proekon*). *Bulletin of Entomological Research* **23**, 549–558.

Harvey ML, Dadour IR & Gaudieri S. 2003. Mitochondrial DNA cytochrome oxidase I gene: potential for distinction between immature stages of some forensically important fly species (Diptera) in Western Australia. *Forensic Science International* **131**, 134–139.

Johnson AP, Wighton SJ & Wallman JF. 2014. Tracking movement and temperature selection of larvae of two forensically-important blow fly species within a "maggot mass". *Journal of Forensic Sciences* **59**, 1586–1591.

Kavazos CRJ & Wallman JF. 2012. Community composition of carrion-breeding blowflies (Diptera: Calliphoridae) along an urban gradient in south-eastern Australia. *Landscape and Urban Planning* **106**, 183–190.

Kurahashi H. 1971. The tribe Calliphorini from Australian and Oriental regions. II. *Calliphora*-group (Diptera: Calliphoridae). *Pacific Insects* **13**, 141–204.

Levot GW. 2003. Insect fauna used to estimate the post-mortem interval of deceased persons. *General and Applied Entomology: The Journal of the Entomological Society of New South Wales* **32**, 31–39.

Mackerras MJ. 1933. Observations in the life-histories, nutritional requirements and fecundity of blowflies. *Bulletin of Entomological Research* **24**, 353–362.

McQuillan PB, Jones AL & Williams H. 1983. Recent studies on sheep blowflies (Diptera: Calliphoridae) in Tasmania. In: *Sheep Blowfly and Flystrike in Sheep*. Proceedings of the second national symposium, University of New South Wales, (ed HW Raadsma), pp. 100–105. Department of Agriculture, Sydney, Australia.

Menzel F, Blaimer BB & Schmitt T. 2017. How do cuticular hydrocarbons evolve? Physiological constraints and climatic and biotic selection pressures act on a complex functional trait. *Proceedings of the Royal Society B: Biological Sciences* **284**, 20161727.

Monzu N. 1977. Coexistence of Carrion-breeding Calliphoridae (Diptera) in Western Australia. PhD thesis. University of Western Australia.

Morris B. 1985. Some South Australian carrion flies of the family Calliphoridae (Diptera). *Western Australian Institute of Technology School of Biology Bulletin* **12**, 67–69.

Morris B. 1993. Physiology and Taxonomy of Blowflies. MAgSc thesis. The University of Adelaide.

Nelson DR & Lee RE Jr. 2004. Cuticular lipids and desiccation resistance in overwintering larvae of the goldenrod gall fly, *Eurosta solidaginis* (Diptera: Tephritidae). *Comparative Biochemistry and Physiology. Biochemistry and Molecular Biology* **138**, 313–320.

Norris KR. 1959. The ecology of sheep blowflies in Australia. In: *Biogeography and Ecology in Australia* (eds A Keast, RL Crocker & CS Christian), pp. 514–544. Springer, Dordrecht.

O'Flynn MA. 1976. A study of blowflies breeding in sheep and in carrion. Department of Parasitology M.Ag.Sc. Thesis, Department of Parasitology, University of Queensland, Brisbane, Australia.

O'Flynn MA. 1980. Studies on blowflies and related flies from Queensland. Ph.D. thesis. University of Queensland.

Palmer DH. 1975. A quantitative survey of the Calliphoridae (Insecta: Diptera) on Carrion. BSc (Hons) Thesis, Australian National University.

Schmidt-Nielsen K. 1997. *Animal Physiology*. Cambridge University Press, Cambridge, United Kingdom.

Wallman JF. 2001a. A key to the adults of species of blowflies in southern Australia known or suspected to breed in carrion [corrigendum in Medical and Veterinary Entomology 16: 223]. *Medical and Veterinary Entomology* **15**, 433–437.

Wallman JF. 2001b. Third-instar larvae of common carrion-breeding blowflies of the genus *Calliphora* (Diptera: Calliphoridae) in South Australia. *Invertebrate Taxonomy* **15**, 37–51.

Wallman JF & Adams M. 1997. Molecular systematics of Australian carrion-breeding blowflies of the genus *Calliphora* (Diptera: Calliphoridae). *Australian Journal of Zoology* **45**, 337–356.

Wallman JF & Donnellan SC. 2001. The utility of mitochondrial DNA sequences for the identification of forensically important blowflies (Diptera: Calliphoridae) in southeastern Australia. *Forensic Science International* **120**, 60–67.

Wallman JF, Leys R & Hogendoorn K. 2005. Molecular systematics of Australian carrion-breeding blowflies (Diptera: Calliphoridae) based on mitochondrial DNA. *Invertebrate Systematics* **19**, 1–15.

Accepted for publication 25 July 2022.