Parasites & Vectors

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Colonisation and temperature-driven variation in life-history traits of the West Nile virus vector *Culex pipiens* at its Northern European limit

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Abstract

Background Mosquito-borne diseases (MBDs) are increasing worldwide driven by factors such as travel, land-use change, global trade, and climate warming. In Europe, *Culex pipiens* sensu lato (s.l.)—the primary vector of West Nile and Usutu viruses—is widespread and an increasing public-health concern with recent detection of WNV in English mosquitoes and ongoing climate change underscoring risks in northern regions. However, data on how temperature affects life-history traits of high-latitude populations remain limited, hindering accurate predictions of vectorial capacity.

Methods We used field-collected larvae in Scotland to establish a colony of *Culex pipiens* and performed its molecular characterisation by PCR, including *Wolbachia* presence. We then assessed the effects of temperature on key life-history traits, including development time, adult longevity, and body size.

Results We successfully established a colony of *Culex pipiens pipiens* infected with *Wolbachia* at its northern range limit. We observed that temperature increase speeds up the embryonic, larval and pupal development while reducing the adult longevity and adult size. Interestingly, this northern colony display the longest adult survival ever described for non blood fed *Cx. pipiens* at all temperature examined.

Conclusions Our findings provide critical baseline data for this northern population and offer methodological guidance for establishing *Cx. pipiens* colonies from field material. These results improve our understanding of thermal sensitivity in Northern *Culex* mosquitoes and will support future modelling of population dynamics and MBD risk under climate change.

Keywords Mosquito, Northern *Culex pipiens*, Life traits, Embryonic development, Larval development, Adult longevity

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Background

The rise, geographic expansion, and re-emergence of mosquito-borne diseases (MBDs) affect all continents, including Europe. These epidemiological changes stem from interacting drivers such as increased human travel, land-use modification, global trade, and climate change [1, 2]. More specifically, climate change-particularly global warming-contributes to the rise in MBDs by (i) promoting the spread of invasive mosquito vectors such as *Aedes albopictus* into new areas [3, 4] and (ii) enhancing the transmission potential of native vector species. Although considerable attention has focused on the risks posed by invasive species, native vectors also require careful investigation, especially given their often-high abundance and the more immediate risks they may present.

Culex pipiens sensu lato (s.l.), one of the most widespread and common mosquito species in Europe, is the primary vector for several important zoonotic viruses, such as West Nile virus (WNV), a virus which has spread and reach its largest geographical distribution ever reported in Europe in 2024 (ECDC). *Cx. pipiens* species consists of two subtypes: *Culex pipiens pipiens* (*Cx. p. pipiens*), which primarily feeds on birds, maintaining virus transmission cycle between birds and mosquitoes, and *Culex pipiens molestus* (*Cx. p. molestus*), which prefers mammals, and is considered as bridge vector transmitting to horses and humans [5, 6]. The recent detection of WNV in British mosquitoes (https://www.gov.uk/government/news/first-detection-of-west-nile-virus-in-uk-mosquitoes), together with the establishment and circulation of Usutu virus (USUV)-another flavivirus transmitted by *Culex* mosquitoes-in England since 2020 [7–9], and its recent detection in two blackbirds on Arran Island, Scotland [10], highlights the growing presence of mosquito-borne flaviviruses in the UK. Combined with the high abundance of *Culex* vector species [11], including in northern regions such as Scotland [12, 13], the presence of migratory bird reservoirs, the increasing geographical proximity of these viruses, and the ongoing effects of climate change, these findings indicate an imminent and continuing risk of *Culex*-borne viruses emergence and spread in the UK [2, 14, 15].

To accurately assess the risk of MBD emergence and spread by natural mosquito populations (*i.e.*, vectorial capacity), and to predict how this risk may change under future environmental conditions, it is essential to investigate how factors such as temperature influence the ecological and mosquito life-history traits that underpin pathogen transmission. Key traits include developmental timing, adult size, adult longevity, and vector competence-the ability of a mosquito to be infected and further transmit a virus to a new host [16]. These traits are major determinants of individual fitness and transmission potential, and each typically follows a thermal performance curve characterised by an optimal temperature and declining performance at temperatures above and below this optimum. Thermal responses can vary substantially among populations of the same mosquito species [17, 18], due to, for example, local adaptation, genetic divergence, variation in microbiota, or the presence of endosymbionts such as *Wolbachia*. Consequently, understanding current thermal adaptation and predicting responses to climate warming requires population-specific data from geographically distinct populations, particularly those distributed across latitudinal gradients [19, 20].

Addressing these questions requires experimental systems that allow environmental conditions to be manipulated under controlled and replicated settings. Although establishing laboratory colonies from field-captured mosquitoes can be challenging [21, 22], such colonies enable controlled, replicated experimental studies that would be difficult or impossible to conduct in the wild or using field-caught individuals. Several *Cx. pipiens* colonies established from different European countries including at its Northern range limit (*e.g.*, Finland, *Cx p. pipiens* [23]; Sweden, *Cx p. molestus* [24]; Germany, *Cx. p. molestus*, Serbia, *Cx. p. molestus* [25, 26]*;* Netherlands, *Cx p. pipiens, Cx p. molestus, Cx. p.* hybrid *pipiens/molestus* [27, 28]*;* England, *Cx p. pipiens, Cx. p. hybrid pipiens/molestus* [29] have facilitated our understanding of how temperature shapes vector competence for arboviruses. Several studies have also assessed the effect of temperature on life-history traits – other than vector competence-in *Cx. pipiens* (Fig. 1 and Table 1) [17, 18, 30–46]. However, these studies are almost exclusively based on temperate to warm-climate populations located in endemic WNV areas (*e.g.*, American, Southern Europe, North African populations located between 30N and 42.6N latitudes), leaving a critical knowledge gap in our understanding of the current thermal biology and adaptations of northern *Cx. pipiens* populations and how they may respond to rising temperatures.

Here, we successfully established a colony of *Cx. p. pipiens* from field-collected larvae in Scotland. We describe the colonisation process in detail and provide a comprehensive molecular characterisation of the colony, including species identification and the detection of the *Wolbachia* endosymbiont in the colony. This work enabled the study of key life-history traits in one of the northernmost *Cx. p. pipiens* colonies to date. Specifically, we examined the effects of temperature on development time, adult longevity, and body size, providing insight into life-history responses near the species' northern range limit. Our findings indicate that these mosquitoes

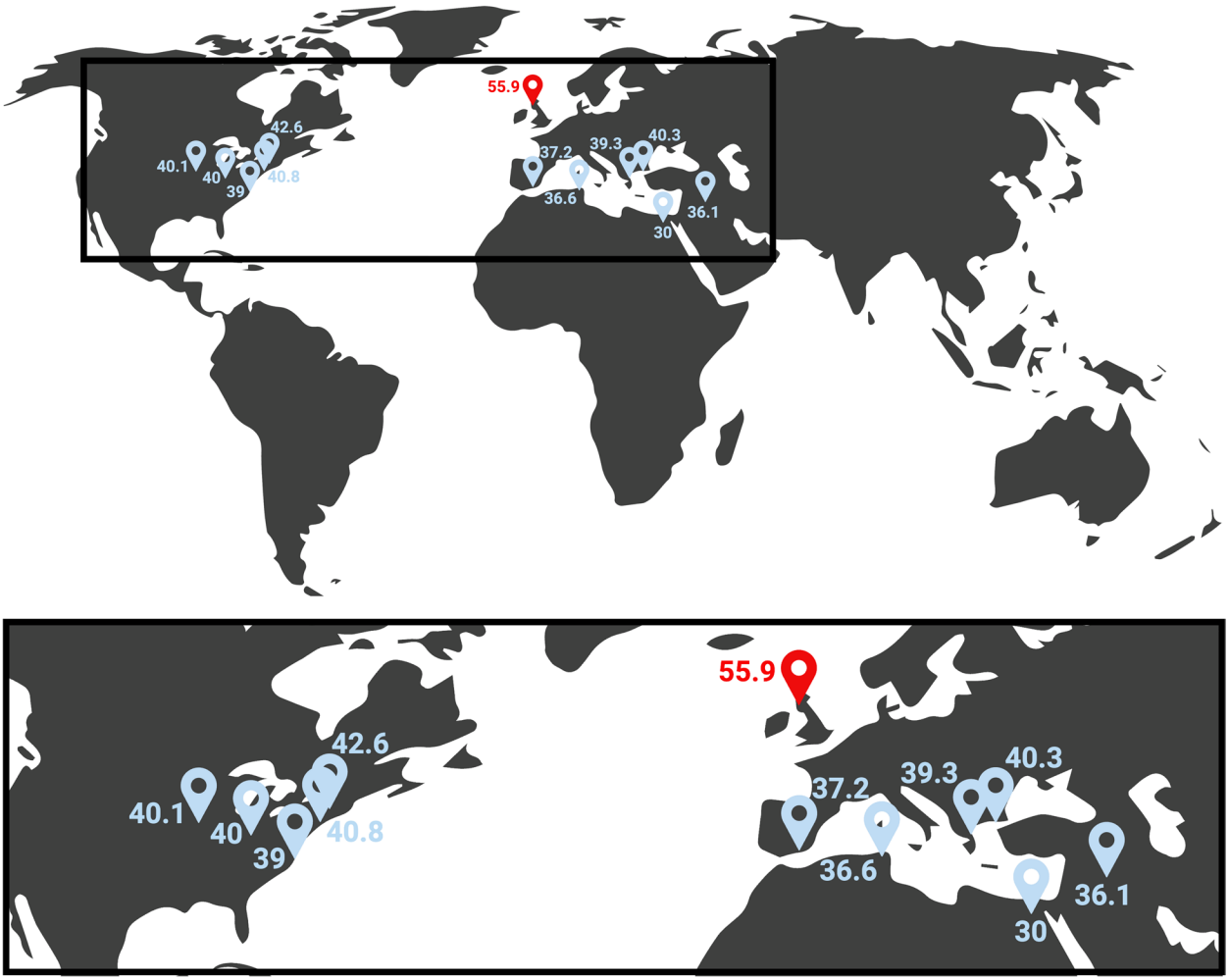


Fig. 1 Origin of *Culex pipiens* populations used for life-history traits studies. Top panel: world map showing the geographic origin, including latitude, of *Cx. pipiens* populations for which survival has previously been analysed and included in our analysis. Bottom panel: enlarged view. Studies were included in the dataset if they measured the longevity of *Cx. pipiens* adults under constant temperature conditions and either measured adult wing length or provided sufficient detail to recover the food provided per larva per day (Supplementary file 1). Each blue mark is a published record; red mark represents the location of the Bearsden colony

exhibit the longest adult survival reported at 18 °C for non blood fed *Cx. pipiens*, consistent with a preference for cooler temperatures and suggesting that this northern *Cx. pipiens* population may be adapted to cooler climatic conditions. These results contribute critical parameters for modelling *Cx. p. pipiens* population dynamics and assessing how this native vector may respond to ongoing climatic change.

Methods

Colony establishment and rearing

Rainwater filled flowerpots of different colours were placed in a garden in Bearsden, Glasgow (55.9032366°N, 4.3266556°W) between the 19th of July and the 8th of August 2023 to attract gravid female mosquitoes. Mosquito egg rafts–*Culex* eggs-were only found in dark red coloured flowerpots (Fig. 2A). After hatching, approximatively 500 larvae were transported to the laboratory (generation F0) in the water they were found in and placed in pans (Fisher-Scientific, ref. 11728511) containing 2–3 L of water, with 200–250 larvae per pan. Larvae were then morphologically identified as *Cx. pipiens/torrentium* (Fig. 2B). Mosquitoes were reared in Panasonic climatic chambers at 20° C, 75% humidity, under a 18 h/6 h inverted light dark cycle, to mimic the duration of the Scottish summer light cycle and allow blood feeding in the dark during the day. Larvae were fed with dry cat food pellets (Friskies) provided ad libitum and changed every 2 days to avoid the buildup of microbial contamination. Using a plastic transfer pipette (VWR,

Table 1 Origin of *Culex pipiens* populations used for life-history traits studies

Publication	Origin (city, country, GPS)	Latitude	Year established	Adult feeding regime
Abouzied, 2017	Cairo, Egypt	30	2012	Sugar crystal + water + blood
Aissaoui et al., 2023	Natural Park El Kala, Algeria	36.6	1995	10% sugar + blood
Alto et al., 2012	Champagne County, Illinois, USA	40.1	Unknown	10% sucrose + water
Andreadis et al., 2014	Chalkidiki, Palaiochori, Greece 40.29N, 23.38E	40.3	2009	10% sucrose
Bianco et al., 2024	Colombus, Ohio, USA (Buckeye strain)	40	2000	10% sucrose
Ciota et al., 2014	Slingerlands, Pennsylvania, USA (colony)	42.6	2002	10% sucrose
Ciota et al., 2014	Takoma Park, Maryland, USA (field)	39	2010	10% sucrose
El-Sayed and El-Bassiony, 2016	Cairo, Egypt	30	2012	10% sugar + blood
Fay et al., 2024	Albany, New-York, USA	42.6	2021	10% sucrose + blood
Fay et al., 2024	Yaphank, New-York, USA	40.8	2020	10% sucrose + blood
Fiorta et al., 2024	Colombus, Ohio, USA (Buckeye strain)	40	2000	10% sucrose + water
Garrigos et al., 2024	Granada, Spain	37.2	2022	10% sugar
Gerberg et al., 1969	Unknown	Unknown	Unknown	10% sucrose
Hamaidia and Soltani, 2021	Natural Park El Kala, Algeria	36.6	1995	10% sucrose
Papadopoulos et al., 2016	Nea Agchialos Magnisias, Greece 39.26N, 22.81E	39.3	2013	10% sugar
Reinhart et al., 2010	Colombus, Ohio, USA (Buckeye strain)	40	2000	Honey + water
Reinhart et al., 2012	Colombus, Ohio, USA (Buckeye strain)	40	2000	Honey + water
Villareal et al., 2025	Ohio, New-York, USA	40	Unknown	10% sugar
Yaba and Al-Muffti, 2024	Center of Erbil-Shanader Park/Soran-Berzewa/ Koysinjaq/Shaqlawa-Aquban, Iraq	36.1	2020–2021	10% sugar + blood
This study	Bearsden, Glasgow, UK, 55.9N, 4.3W	55.9	2023	10% sucrose

Study references, *Culex pipiens* s.l. geographical origin (shown on Fig. 1), latitude, year of establishment and adult feeding regimes. Study details are given in Supplementary data 1

(See figure on next page.)

Fig. 2 The Scottish *Culex* colony *is Culex pipiens pipiens*. **A** Picture of some egg rafts from which the Bearsden colony was established. **B** Picture of a F0 larvae identified as belonging to the *Culex pipiens* complex **C**–**D** PCR-amplicon size assay to identify *Culex* species and biotypes. **C** Using *Culex pipiens* and *Culex torrentium* specific primers, a PCR fragment close to 600 bp was obtained from amplification of genomic DNA extracted from five males and five females sampled from the colony showing that these mosquitoes are *Culex pipiens*. **D** Using *Cx. p. pipiens* and *molestus* specific primers, a PCR fragment close to 200 bp was obtained from amplification of the genomic DNA from the same mosquitoes showing that these mosquitoes are *Culex pipiens* biotype *pipiens*

ref. 612-6803), pupae were transferred to Gallipots (VWR, ref. 216-1021) containing 40 mL of water and placed in a 30×30 × 30 cm Bugdorm cage (Watkins & Doncaster, ref. E6099).

Emerging adult mosquitoes were separated into two cages and maintained on a 10% (w/vol) sucrose solution changed every 4–5 days. Females were fed multiple times with 2×3 mL of heparinised blood (Envigo, UK) for 45 min using a 37 °C Hemotek system (Hemotek Ltd, Blackburn, UK). Feeding was the most challenging step, requiring testing varying conditions and optimisations (Supplementary Table 1). Exploratory observations were done during the first stages of colonisation to assess which conditions were optimal for blood feeding. Due to the limited number of mosquitoes, the nature of colonisation, and the need to allocate most individuals to colony establishment, these optimisation tests could be performed only once. Initial attempts to compare blood feeding rates between mosquitoes that were sugar-starved 24 or 48 h before the blood meal resulted in a substantial mortality of males in the 48-h treatment (data not shown). Thus, we optimised blood feeding rates in mosquitoes that had been sugar-starved for no more than 24 h prior to feeding, with water provided on a cotton ball to prevent desiccation. To explore optional conditions for blood feeding, blood meals were presented to females at different temperatures (37 °C and 40 °C), using blood from different host species (rabbit or chicken), different membranes (artificial collagen membrane-Hemotek Ltd, ref. MEM5 or hog intestine-Tongmaster, Amazon), and with different attractants (nonanal-Sigma-Aldrich, ref. N30803, pheasant, duck or grouse scented sticks-TopDog, Amazon) placed on cotton next to the feeder (nonanal) or on artificial membranes (scented sticks). Interestingly, during the first 23 days post-emergence, only few females (1 to 5) successfully blood-fed,

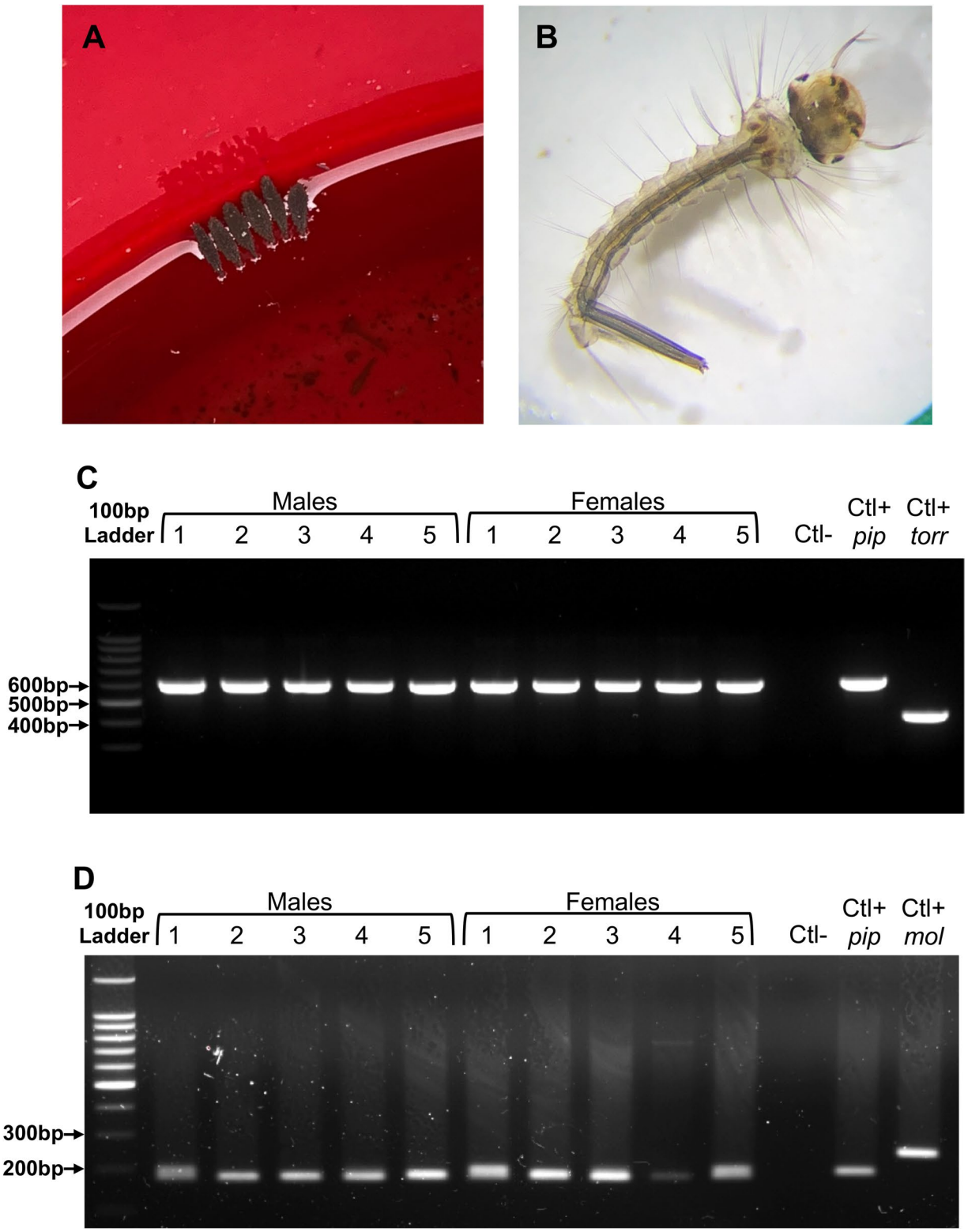


Fig. 2 (See legend on previous page.)

regardless of the blood source, temperature, additive or attractant used (Supplementary Table 1). High temperatures (40 °C) tended to coagulate the blood quickly, making feeding difficult after 15–20 min. Although we tested several combinations of blood sources, temperatures, blood additives, attractants and feeding membranes (Supplementary Table 1), we did not identify any blood additive or attractant that significantly improved feeding success. The first successful blood meal by F0 females occurred 24 days post-emergence and coincided with the use of hog intestine as a feeding membrane. Some rare feeding event on rabbit blood were observed only when hog intestine was used, and feeding success increased further with a chicken blood–hog intestine combination. From F1 onward, females younger than 20 days post-emergence did not feed successfully under any conditions tested. At 20–21 days post-emergence, feeding rates of 30–64% (data not shown) were achieved using chicken blood with hog intestine, whereas feeding on rabbit blood or artificial membranes was rarely observed and consistently lower. For egg laying, transparent plastic cups (G&S Packing UK LTD, Amazon), covered or not with red paper tape (VWR, ref. 817-1525), and filled with 250 ml of dechlorinated tap water (tap water left for at least two days at room temperature, RT), were placed in cages from day three to six post blood-feeding. Eggs were further transferred in pans containing dechlorinated tap water and with dry cat food pellets.

Using our optimised conditions-blood feeding after 20 days using chicken blood and hog intestine, and red laying cups-we successfully established a *Culex* s.l. colony (hereafter named Bearsden colony). From F2 onward, the colony reached enough individuals to remain stable and F3 was further transferred and kept in an insectary at 24° C, 75% humidity, under a 18 h/6 h inverted light dark cycle, including 1 h of dusk and 1 h of dawn.

Molecular identification of *Culex* species and biotype and detection of *Wolbachia* presence

Culex species/biotype identification and testing for *Wolbachia* presence was conducted through genotyping at different generations (see Results). DNA was extracted from whole adult mosquitoes using the REDExtract-N-Amp Tissue PCR Kit (Sigma-Aldrich, ref. XNAT2) according to manufacturers' instructions. Each mosquito was extracted in 125 µL of extraction buffer (100 µL of Extraction solution mixed with 25 µL of tissue preparation buffer) and mashed with a tip without expelling the buffer. Once the mosquito was ground, buffer was released from the tip, and the sample was incubated at 55°C for 10 min then at 95°C for three min. Finally, 100 µL of neutralisation solution were added. Genomic DNA (gDNA) abundance was measured using Nanodrop One (Fisher Scientific, ref ND-ONE-W) and diluted to reach 50 to 100 ng of gDNA/µL. For PCR amplification, 10 µL of REDExtract PCR mix were mixed with 0.8 µL of each primer, 1 µL of template and volume adjusted to 20 µL using nuclease free water. Primers and PCR parameters used for species identification (pipCQ11R, molCQ11R, CQ11F2 for *pipiens*/*molestus* and ACEpipF, ACEtorrF, B1246sR for *pipiens*/*torrentium*) and for testing *Wolbachia* presence (wsp81F, wsp691R) are described in Supplementary file 2. The amplicons were analysed by electrophoresis by loading 10µL of the PCR product on a 2% agarose gel with ethidium bromide (EtBr, Sigma-Aldrich, ref. 1.11608), including a 100 base-pair (bp) ladder to control product size. Results were visualised using the Biorad GelDoc Go Imaging System.

Identification of *Wolbachia* species

When the colony was established (generation F2), gDNA was extracted from 5 ovary pairs using DNAzol reagent (Invitrogen, ref. 10503027) following manufacturers' instructions and quantified using NanoDrop One. gDNA was diluted to 50 ng/µL using NaOH 0.8 M and 1 µL was used for PCR amplification using the KOD polymerase (Sigma-Aldrich, ref. 71086-5) by mixing 25 µL of KOD mix with 21 µL of water and 1 µL of each primer (w16SF, w16SR, Supplementary Table 2). The PCR product was visualised on a 1.5% agarose gel containing EtBr and subsequently purified using QIAquick Gel Extraction Kit (Qiagen, ref. 28704). After nanodrop quantification, 75 ng of the PCR product were used for ligation in pJET vector (Thermo Scientific, ref. K1231) following manufacturers' instruction in 20 µL total volume. Two µL of the ligation were used for transformation in Sure2 super competent cells following manufacturers' instruction (Stratagen, ref. AGLS200152) and 100 µL of the transformation were plated on LB-ampicillin agar plate incubated overnight at 37° C. Ten bacterial colonies were screened by PCR using w16SF and w16SR primers as described previously. One of the positive colonies was amplified overnight at 37°C in LB broth and the vector was purified with the QIAprep Spin Miniprep Kit (Qiagen, ref. 27104). Finally, the insert was sequenced using pJet-reverse and T7-forward primers (Eurofins). The sequence was analysed using the Snapgene software (GSL Biotech LLC, version 8.2.0) and used to blast NCBI to identify the *Wolbachia* species present in the colony. Blast showed 100% of identity with *Wolbachia pipientis 16S* rRNA from *Cx. pipiens* s.l. (X61768) collected in Champaign, IL [47].

Detection of *Wolbachia* by in situ hybridisation

Fluorescent in situ hybridisation (FISH) was carried out according to *Ant* et al., *2018* protocol [48]. Two fluorescent probes-published in *Osborne* et al., *2012*, which

have 100% identity to the *Wolbachia 16S* identified in our colony-were used to visualise the *Wolbachia* 16S RNA: 5′-CTTCTGTGAGTACCGTCATTATC-3'[Atto488] and 5′-AACCGACCCTATCCCTTCGAATA-3′ [Atto488] (Sigma-Aldrich). Tissues were dissected in RNase free PBS with 0.05% of Triton X-100, fixed for 20 min at room temperature in PBT (RNase free PBS with 0.1% Triton X-100) and washed three times with PBT at RT. Prehybridisation was carried out for 30 min at 37° C and probe hybridisation over night at 37° C. Tissues were washed three times for 10 min at RT with PBT, and DAPI staining was carried out for 1 h at RT in PBT. After two washes of 10 min at RT, tissues were mounted in Ibidi mounting medium (Ibidi, ref. IB-50001) between slide and coverslip. Tissues were imaged using a Zeiss confocal 710 and pictures were processed with Fiji software [49].

Analysis of developmental timing and adult longevity

To determine development duration, adult longevity, and the effect of temperature, experiments were conducted in climatic chambers under an inverted 18 h/6 h light dark cycle and 75% humidity, at different temperatures: 18 C, which is the current average daytime temperature during summers in Southern Scotland; 24 C, which may be reached by 2070 in the RCP8.5 high emission scenario; and 28 C, predicted to be a temperature occurring more frequently during summer heatwaves [50]. In addition, this temperature range was chosen to be able to compare life traits with as many studies as possible, with most published studies ranging between 18 and 28 C. Egg hatching was checked every four hours to determine the duration of the embryonic development (time from laying to hatching). Duration of the larval and pupal stages, as well as the adult survival were monitored in quadruplicates using larvae hatched from the eggs used to measure embryonic stage duration. For each temperature, four replicates were generated by collecting 4 times 100 newly hatched first instar larvae (all hatched during the same time period and from eggs reared at the same temperature, less than four hours old) with a plastic transfer pipette and transferring them into four pans containing 2 L of dechlorinated tap water and fed with a cat food pellet (1 pellet, 269.8±8 mg, from 1st and 2nd larval stages and 2 pellets for 3rd and 4th larval stages). Food was changed every 2 days. Larvae were kept under constant temperatures (18° C, 24° C or 28° C). Pupariation was monitored twice a day, at morning (9am) and afternoon (4 pm) time to determine the duration of the larval stage. Pupae were transferred to Gallipots and placed in a pan covered by a net at the same temperature as the larvae. Adult emergence date and sex were recorded once a day at 1 pm. For each of the four replicate and at the three temperatures, 30 newly emerged males were transferred in a carboard box (Street Food Packing, ref. SOUP32 and SOUPLID32) covered with a net and 30 females were transferred to a 17.5×17.5 × 17.5 cm Bugdorm cage (Watkins & Doncaster, ref. E61010M) to which 10 males were added. Adults were maintained on a 10% (w/vol) sucrose solution, changed every 2–3 days, and kept in the same conditions as the previous stages to follow survival. Adult survival was recorded every 2–3 days until all mosquitoes were dead.

Wing measurements

Dead adult mosquitoes from the survival analysis were kept frozen at − 20° C in falcon tubes. Wings were dissected and placed between slide and coverslip in Ibidi mounting medium. Pictures were taken using a binocular M60 microscope equipped with a camera (Leica). Lengths were measured from the proximal end of the axillary cell of the wing to the distal end of the posterior vein of the first submarginal cell.

Statistical analysis

Influence of temperature on embryonic development duration was analysed using Kruskal–Wallis test followed by post hoc corrected Dunn's test. Influence of temperature on larval/pupal stage duration was analysed using generalised linear mixed effects models (GLMM) including temperature as a fixed effect and replicate as a random effect using a log-normal distribution with a log link using the package glmTMBB [51]. Model assumptions (*e.g.*, residuals, overdispersion) were checked using DHARMa and marginal means were estimated using the emmeans package [52, 53]. Tukey post hoc test was used to get multiple comparisons. Analysis was conducted using R version 4.5.0 [54]. Influence of temperature and sex on adult survival was analysed with Graphpad Prism version 10.6.1 using a Kaplan–Meier model. Statistical significance between temperatures and sexes was evaluated using Mantel-Cox Log rank test. The effect of temperature, sex and the interaction effect of temperature and sex on mosquito wing length was tested for using a GLMM, with temperature and sex as fixed effects and replicate as a random effect using the package lme4 [55]. Model assumptions (*e.g.*, residuals, overdispersion) were checked using DHARMa and marginal means estimated as before. A post-hoc Tukey test was used to compare the differences between sexes at each temperature and compare the differences between temperatures for each sex. Significant p values are indicated in the respective figure legends. The time until 50% percent adult mortality observed in this study was compared to a range of published estimates of the same trait from laboratory colonies of *Cx. p. pipiens* over a similar range of temperatures (References in Supplementary data 1). Studies

were identified by searching Web of Science and Google Scholar using the terms "*Culex pipiens*" & "temperature". Studies were included in the dataset if they measured the longevity of *Cx. pipiens* adults under constant temperature conditions and either measured adult wing length or provided sufficient detail to recover the food provided per larva per day. From these initially identified studies we performed a snowballing step to identify additional sources either from reference lists or other studies conducted by the same authors.

Results

Establishment and molecular characterisation of a *Wolbachia*-infected *Culex pipiens pipiens* colony

We successfully established a colony of mosquitoes from field-collected egg rafts/larvae in Scotland morphologically identified as belonging to the *Culex pipiens* complex (Bearsden colony, Material and methods, Fig. 2A, B). This complex includes cryptic forms /species that are morphologically indistinguishable, *i.e., Cx. p. pipiens*, *Cx. p. molestus* and *Cx. torrentium* [56]. To identify the population colonised, we first screened five F0 males and five F0 females. PCR amplification of gDNA demonstrated that they were all *Cx. p. pipiens* (Fig. 2C, D). We then isolated the egg rafts from the first generation (F1), of which 22 hatched and successfully developed into adults. Species and biotype were then determined by PCR using one adult originating from each of the individual raft. PCR analysis showed that the 22 rafts were belonging to the *Cx. pipiens* biotype *pipiens* (data not shown). Adults were thus collected and grouped into cages for further amplification. Molecular analysis of a further 19 rafts from the second generation (F2) confirmed that the Bearsden colony was *Cx. p. pipiens*.

We further assessed the presence of *Wolbachia* in these mosquitoes. This intracellular bacterial symbiont is known to be present in many insect species where it modulates the longevity, metabolism, behaviour, stress response and pathogen resistance of infected hosts [57]. PCR was carried out on the same samples used for species/biotype identification described above. All the adult tested were positive for *Wolbachia* (Fig. 3A). Further sequencing analysis confirmed the presence of *Wolbachia pipientis*. The distribution of *Wolbachia* in mosquito tissues was further examined using in situ hybridisation targeting the 16S ribosomal RNA (Fig. 3B–E). *Wolbachia* was detected in the ovaries mainly in the nurse cells (Fig. 3B), the central brain (Fig. 3C, Supplementary File 1) and the fat body (Fig. 3E) and to a lesser extent in some stellate cells of the Malpighian tubules (Fig. 3D), demonstrating a wide distribution of *Wolbachia* across tissues.

Effect of temperature on developmental timing

To date, studies analysing *Cx. pipiens* life-history traits other than vector competence have been largely limited to populations from warm or temperate regions (Fig. 1, Table 1). Taking advantage of our colony and to evaluate the effect of temperature on the life traits of a more Northern *Cx. pipiens* population, we first analysed the developmental timing from embryonic stage to adult stage at three different temperatures (18 °C, 24 °C, 28 °C, Fig. 4A). The time of embryonic development was very uniform among egg rafts at each temperature with 100% of egg rafts hatching within the same 4-h window (Fig. 4B). At 18 °C, hatching occurred between 67 and 71 h post egg laying, at 24 °C this time was reduced to 39 to 43 h, and at 28 °C hatching occurred between 33 and 37 h post oviposition (Fig. 4B). These results demonstrate a significant effect of temperature on embryonic developmental speed ($p < 0.0001$) with embryonic durations differing significantly between temperatures.

The median larval developmental time-defined as the time at which 50% of larvae have transitioned to pupae—was also significantly influenced by temperature (Fig. 4C, D, $p < 0.001$). At 18 C, the median developmental time was 422.3±11.5 h post-hatching (17.6 days), compared with 252±7 h post-hatching (10.5 days) at 24 C, and 208±6.3 h post-hatching (8.7 days) at 28 C showing that an increase in 10 degrees can reduce the larval development time by more than a half (Fig. 4D).

The estimated time at which 50% of larvae have transitioned to the adult stage was also significantly impacted by temperature (Fig. 4E, F, $p < 0.001$) with 569±8 h post-hatching (23.7 days) at 18 C, 329.8±5 h post-hatching (13.7 days) at 24 C, and 287±4.5 h post-hatching (12 days) at 28 C. Altogether, these results show that the development duration of Scottish *Cx. pipiens* is highly temperature dependent, varying by nearly twofold between the highest (28 °C) and lowest (18 °C) temperatures tested.

Effect of temperature on adult longevity

We further assessed the survival of the *Cx. p. pipiens* adults using mosquitoes emerged from the developmental timing experiments across a similar range of temperatures (18 C, 24 C and 28 C, Fig. 4A and Fig. 5, Supplementary File 2). Females significantly survived longer than males at all temperatures (Supplementary File 2, $p < 0.0001$). Median adult longevity at 18 C was higher than anticipated, with median survival of 87.5±17.1 days post-emergence (roughly 3 months) for males and 126.6±27 days (4.2 months) for females, and maximum survival of 165 days for males and 179 days for females, equivalent to almost 6 months (Supplementary

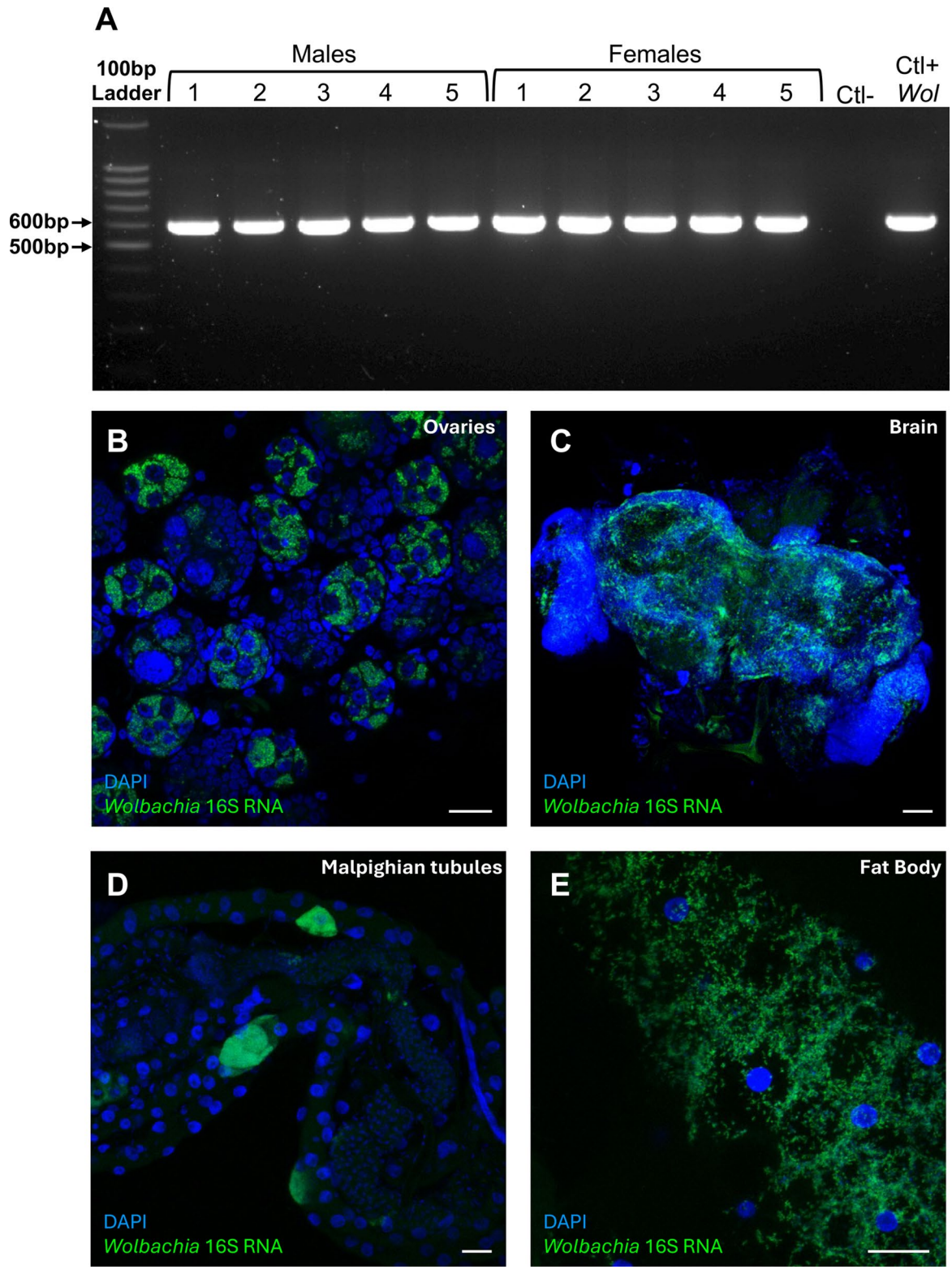


Fig. 3 The Scottish *Culex pipiens pipiens* are infected by *Wolbachia*. **A** *Wolbachia* detection by PCR in five males and five females sampled from the colony. *Wolbachia* detection in non blood fed females using FISH (*Wolbachia 16S* probe, green) showing infection in the ovaries **B**, the brain **C**, the stellate cells from Malpighian tubules **D** and the fat body **E**. Cell nuclei were stained with DAPI (blue). Scale bars are 20 µm for **B** and **E** and 50 µm for **C** and **D**

File 2A and Fig. 5). Increasing the temperature by 6 degrees (18 to 24 C) led to a dramatic reduction of survival by around 50%, with a median at 50.7±9 days post-emergence (maximum 86) for males and 65.7±5.6 days (maximum 93) for females (Supplementary File 2B and Fig. 5). Finally, at 28 C, the median survival dropped to 26.7±4.3 days (maximum 56) for males and 38.7±10.5 (maximum 71) for females (Supplementary File 2C and Fig. 5). For both sexes, temperature had a significant effect on survival, with each temperature group differing from the others and adult survival declining significantly as temperature increased (Fig. 5).

Effect of temperature on mosquito size

Temperature strongly influences mosquito size in mosquitoes, including in *Culex* species, which in turn can affect development, longevity, reproductive output, and ultimately the species' potential as a disease vector [30, 36, 58]. To assess the effect of the temperature on the adult size of Scottish *Cx. p. pipiens* mosquitoes, we measured the length of the wings from both adult males and females at 18 C, 20 C and 28 C (mosquitoes used in adult longevity experiment, Figs. 4A and 6A). At all temperatures, wings were significantly smaller in males compared to females (Fig. 6B). For both males and females, wings were significantly smaller at higher temperatures (Fig. 6B).

Discussion

In this study, we successfully established the first *Cx. pipiens* s.l. colony derived from field-caught larvae in Scotland and conducted molecular characterisation confirming the colony as *Cx. p. pipiens* infected with the endosymbiont *Wolbachia*. Many studies have analysed the effect of temperature on mosquito development and population dynamics [58, 59], since quantifying the precise relationships between temperature and life-history traits is essential to understand their effect on the efficiency of MBD transmission, *i.e.*, vectorial capacity. Equally important is directly comparing how medically important mosquito populations from different geographic origins respond to temperature, as this allows to identify local adaptation and better predict how climate change may alter vectorial capacity and patterns of disease transmission [60, 61]. Here, we analysed key life-history traits under three temperature regimes (18 C, 24 C, and 28 C) which revealed marked thermal sensitivity in this Northern *Culex* population. Higher temperatures accelerated embryonic and larval development, whereas cooler temperatures substantially increased adult survival, with the longest recorded longevity for sugar fed non-diapausing *Cx. pipiens* to date (Table 2 and Fig. 7).

Efforts to better understand mosquito biology are strongly supported by studies using mosquito colonies under laboratory conditions. Although colonisation and laboratory settings cannot fully capture the complexity of natural field conditions, for various reasons including potential reductions in population genetic diversity during colony establishment, inherent to most laboratory colonies established from a limited number of founders, they are essential for experimental work that cannot be achieved using field-collected mosquitoes alone, particularly when sufficient mosquito numbers, replication, and statistical power are required. However, the colonisation process can be difficult for several reasons including poor larval survival, mating success, low fecundity and shorter survival under laboratory conditions. In our conditions, the blood feeding step has revealed to be the most challenging step, as previously described [62, 63]. While our *Culex* colony showed a clear preference for natural membranes (*e.g.*, hog intestine) over artificial ones, there is no consistent trend in *Cx. pipiens* rearing methods across studies; some laboratories use artificial membranes, whereas others use natural membranes. Historically, natural membranes (animal skins, intestines, sausage casings) have been widely used, especially for mosquito strains or species with feeding reluctance, because they mimic host skin more closely. Natural membranes may indeed offer more realistic tactile and chemical cues, which can improve feeding success-but they can be harder to source, and may require more preparation (*e.g.*, cleaning). Our results also suggest that the colonised mosquitoes display a preference for chicken blood over rabbit blood. This is consistent with published studies showing that when using different blood sources (avian vs mammalian) under artificial feeding (membrane or

(See figure on next page.)

Fig. 4 Effect of temperature on developmental time. **A** Schematic of the experimental procedure used to assess the developmental timing. Recording steps are highlighted in red. α, β, and γ are the duration of the different developmental stages analysed. **B** Duration of embryogenesis, α. Numbers of egg rafts analysed shown above graph bars. Because hatching times were the same for all egg rafts at a given temperature, the error bars are not visible. Kruskal–Wallis test (general effect of temperature, $p < 0.0001$) followed by corrected Dunn's test for multiple comparison. 18 C vs 24 C $^{**}p = 0.0012$, 18 C vs 28 C $^{****}p < 0.0001$, 24 C vs 28 C $^{***}p = 0.0005$. **C** Time of larval to pupal transition in percentages, β. **D** Median duration of the larval stage, β. GLMM, $^{***}p < 0.001$. Tukey multiple comparisons, $^{****}p < 0.0001$. **E** Time of pupal to adult transition in percentages, $\beta + \gamma$ **F** Median time to adult emergence, $\beta + \gamma$. GLMM, ***p < 0.001. Tukey multiple comparisons, $^{****}p < 0.0001$. Colour coding: 18 C (blue), 24 C (orange) and 28 C (red). Error bars shown represent SEM

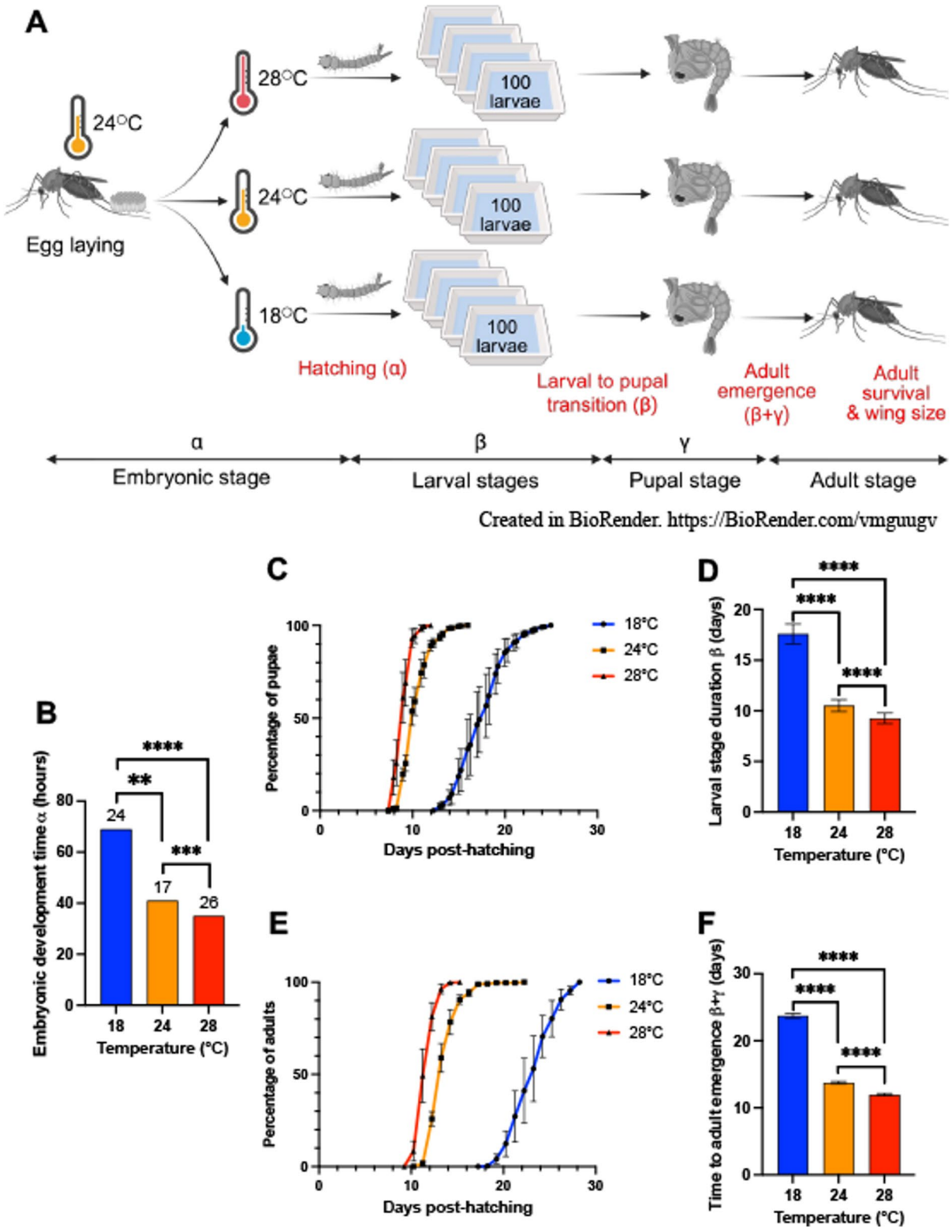


Fig. 4 (See legend on previous page.)

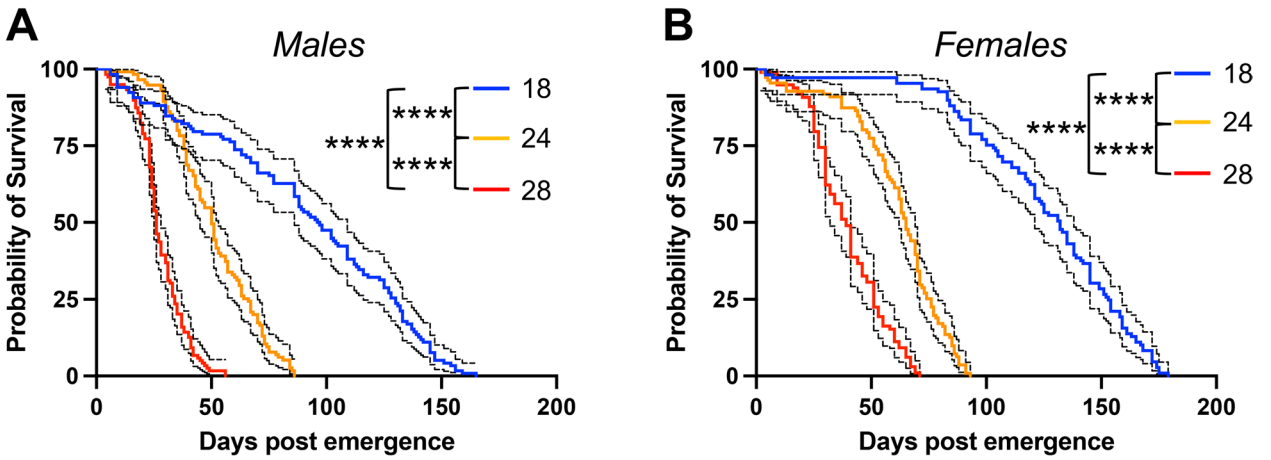


Fig. 5 Effect of temperature on adult longevity. Comparison of survival of males **A** and females **B** at 18 C, 24 C and 28 C. Black dotted lines represent 95% confidence intervals. Mantell-Cox log-rank test was used to compare survival curves between groups and are shown on the graph legends (****p-value < 0.0001)

other methods), *Culex* mosquitoes showed differences in feeding activity, fecundity, and egg production, often favouring avian blood [64]. While the *pipiens* biotype is considered to be primarily ornithophilic, in contrast to the mammalophilic *molestus* biotype [6], the different attraction is mediated by skin/body odours, not the smell of blood per se. It is possible that when using an artificial feeding system, females probe through the membrane (which could not be observed here as feeding in the dark) and can taste blood as previously demonstrated in *Aedes aegypti* [65]. This may contribute to recognising species-specific differences in blood composition and inform feeding decisions, such as aborting a blood meal if it is unsuitable for optimal egg development or continuing to feed when the blood is adequate. Interestingly, some human-directed host-seeking behaviour was observed in the laboratory, indicating that this population may display a degree of opportunistic feeding. While the dichotomic feeding preferences between *pipiens* and *molestus* are supported by studies in *Culex* originating from the United States [66–68], analysis of blood meals in wild European mosquitoes are less clear, showing no difference in proportion between *pipiens* and *molestus* fed on bird and mammals [69, 70]. Since *Cx. p. pipiens* is one of the most widespread vectors in Europe, its role as a bridge vector should not be neglected [71].

Our observations also suggests that ageing may contribute to an increased likelihood of blood feeding in this colony with best feeding rates obtained from approximatively 20 days post emergence. While this behaviour may reflect an intrinsic characteristic of this northern population, there is no clear evidence in the literature that age alone enhances blood-feeding rates under laboratory conditions. Since blood feeding in this species generally occurs earlier in laboratory conditions (*e.g.*, [72]), this delayed pattern may be indirectly driven by other factors, particularly insemination status. Mating is known to increase blood-feeding propensity in several mosquito species, including *Culex* species [73]. Achieving successful mating can be challenging in the laboratory, as some species require for their mating behaviour specific environmental cues-such as light, airflow, and acoustic signals00–and sufficient flight space [6, 21, 74], that can be difficult to reproduce in standard insectary cages. While we used a light programme mimicking dusk and dawns (*e.g.*, crepuscular light), it is possible that mating success in our *Cx. p. pipiens* colony is limited by sub-optimal laboratory rearing conditions, such as cages not large enough for optimal swarming and mating, resulting in a delayed blood feeding propensity. While aging females slightly longer to obtain satisfactory blood feeding rates have not been an obstacle to establish and maintain this colony, testing these factors (*e.g.*, cage size and insemination status) may help with establishment of some colonies or when required to feed females earlier.

Our data demonstrate that the colonised *Cx. p. pipiens* are infected with the *Wolbachia* bacterial endosymbiont. This infection is consistent with the high prevalence reported in natural populations of *Cx. pipiens* s.l. [75–79]. We demonstrated that *Wolbachia* is present in the ovaries, the Malpighian tubules, and the fat body, consistent with previous studies in *Cx. pipiens* finding high densities in reproductive tissues and medium to low densities in various somatic tissues, which can vary by *Culex* population and *Wolbachia* strain [80]. However, this is the first report of *Wolbachia* presence in the brain of naturally infected adult female mosquitoes. The most convincing evidence for neural localisation comes from

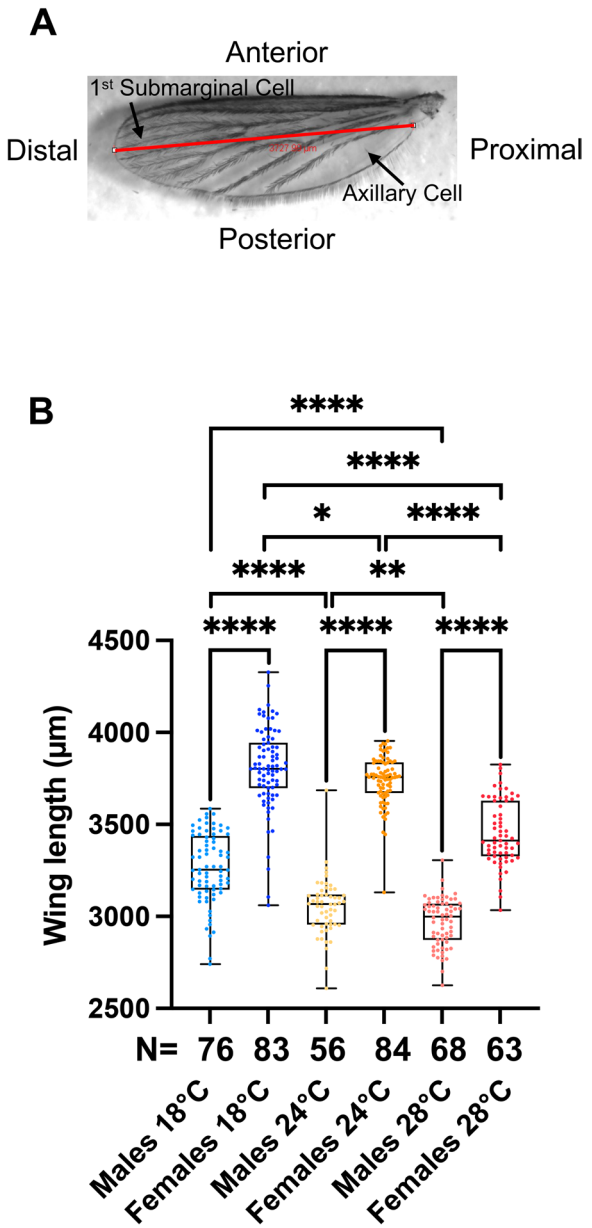


Fig. 6 Effect of temperature on wing size. **A** Example of wing measurement. Measurements were taken between the proximal end of the axillary cell of the wing to the distal end of the posterior vein of the first submarginal cell (red line). **B** Male and female adult wing length measurements in µm at 18 C (blue), 24 C (orange) and 28 C (red). Box plots display the minimum, first quartile, median, third quartile, and maximum relative expression levels. GLMM was used to analyse the effect of sex and temperature on wing size. Main effect of temperature $p < 0.0001$; main effect of sex $p < 0.0001$; interaction effect of temperature and sex $p < 0.0001$; Tukey multiple comparisons, *$p = 0.0432$, **$p = 0.0240$, ****$p < 0.0001$

studies in *Drosophila*, where *Wolbachia* concentrate in the adult central brain with low density in the optic lobes [81], as observed in our *Culex* colony. Interestingly, in the fruit fly, *Wolbachia* infection influences various behaviours including sleep, learning and memory, mating, feeding or aggressiveness [82, 83], but no direct link with *Wolbachia* presence in the brain could be demonstrated. A recent report has also shown that brain-localised *Wolbachia* promote female receptivity potentially by modulating neuronal signalling [84]. It would be interesting to examine the presence of *Wolbachia* in the brain in other *Culex* mosquito strains/species and explore whether this could affect the behaviour of mosquitoes as well.

In this study, to inform on the effect of temperature on developmental timings and adult survival, we characterised the duration of each life stage at three different temperatures (summarised in Table 2). Our findings show that temperature can considerably speed up the development at all stages and adult aging as previously reported in most mosquito species, including *Culex* [30]. Developmental times were similar to the ones already described for other *Cx. pipiens* populations-with shortest development times at 2.3–10 days at temperatures from 30 to 32.5 °C, and longest development times at 25–50 days from 15 to 20 °C [30]. The temperature-size rule (TSR) states that there is an inverted relationship between the developmental temperature—and therefore a positive relationship between the developmental time—and the final size of arthropods [85]. Our data demonstrate that our *Cx p. pipiens* colony follows the TSR, displaying bigger sizes at lower temperatures, as already evidenced in different mosquitoes including *Culex* and *Aedes* [36, 86].

In contrast, when comparing the survival of females to survival analyses previously published for *Cx. pipiens* (Fig. 7), this revealed that our colony exhibited the highest longevity compared to previously described populations at each temperature and independently of the adult feeding status [30, 34, 36]. Comparisons across these studies suggest that blood feeding reduces lifespan in *Culex*, consistent with a trade-off between mosquito nutrition/reproduction and longevity previously described in some studies (*e.g., Aedes aegypti* [87]). Whereas the longest adult survivals previously reported for sugar fed *Cx. pipiens* are approximately 120 days at 18–20 °C, sugar fed females from our colony exhibited an exceptional lifespan of up to six months at 18 °C, the current average temperature during summer in Scotland. Our survival analyses also revealed a pronounced reduction in adult survival at warmer temperatures, median survival decreased by around 50% at 24 °C and by more than 70% at 28 °C relative to 18 °C. Earlier studies typically showed an inverted U-shaped relationship between temperature and survival in *Cx. pipiens*, with optimal development occurring near 25 °C [30] (Fig. 7 and Supplementary File 1). Our colony appears to exhibit a preference for cooler temperatures compared to every other *Cx. pipiens* analysed so far,

Table 2 Summary of the Bearsden colony life traits

Temperatures	18 °C		24 °C		28 °C	
Embryonic development duration (hours/days)	67 to 71/2.79 to 2.96		39 to 43/1.62 to 1.79		33 to 37/1.37 to 1.54	
Time of 50% larval to pupal transition (hours/days ± SEM)*	422.3 ± 11.5/17.6 ± 0.48		252 ± 7/10.5 ± 0.29		208 ± 6.3/8.7 ± 0.25	
Time of 50% adult emergence (hours/days ± SEM)*	569 ± 8/23.7 ± 0.33		329.8 ± 5/13.7 ± 0.21		287 ± 4.5/12 ± 0.19	
	Males	Females	Males	Females	Males	Females
Adult longevity in days (50% survival ± SEM/maximum survival)**	87.5 ± 17.1 /165	126.6 ± 27/179	50.7 ± 9 /86	65.7 ± 5.6/93	26.7 ± 4.3/56	38.7 ± 10.5 /71
Adult average wing size in µM (mean ± SEM)	3415 ± 38.21	3790 ± 27.18	3052 ± 20.73	3733 ± 17.15	2970 ± 15.72	3452 ± 20.70

Developmental timing, adult longevity, and wing size of the Bearsden colony at three temperatures

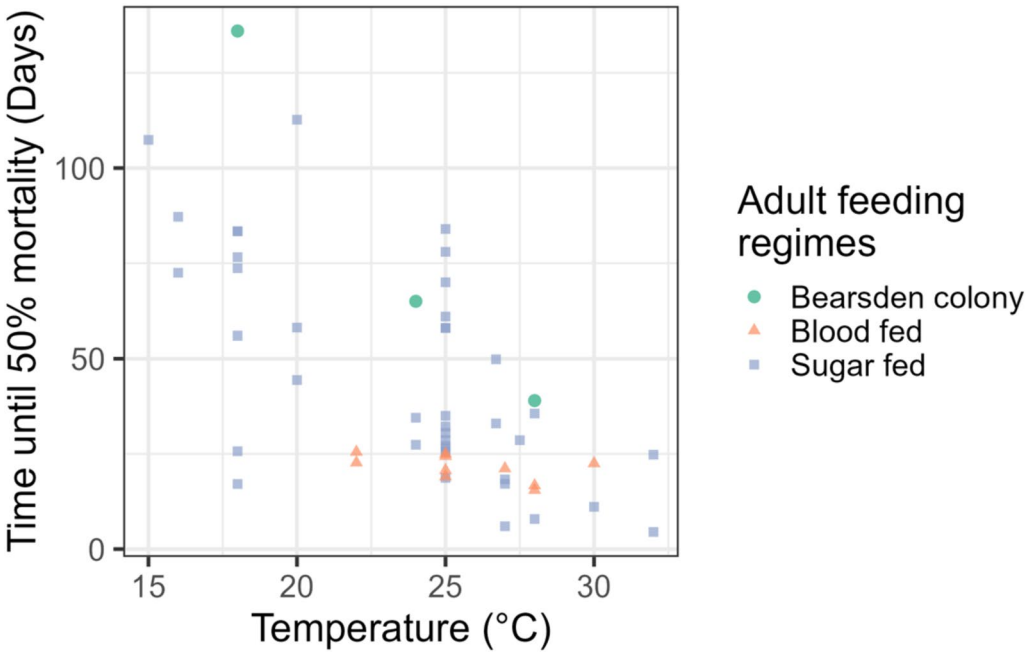


Fig. 7 Comparative analysis of female adult *Cx. pipiens* longevity at different temperatures. Time for 50% mortality recorded in the literature (Supplementary data1) for female adult *Cx. pipiens* at different temperatures. Blue squares represent data from sugar fed adults, red triangles represent data from blood fed mosquitoes and green circles represent data from the Bearsden colony (sucrose fed, this study). Studies were included in the dataset if they measured the longevity of *Cx. pipiens* adults under constant temperature conditions and either measured adult wing length or provided sufficient detail to recover the food provided per larva per day

though survival analyses at more temperatures, especially cooler ones, would be needed to establish a thermal performance curve and confirm this hypothesis. It is notable that our colony represents the northernmost population studied to date (Fig. 1 and Table 1), indicating that Scottish *Cx. pipiens* may be adapted to the cooler climatic conditions of their local environment. Thermal responses of life-history traits-other than vector competence-have been documented in *Cx. pipiens* and other mosquitoes [19], with populations from different climatic regions showing distinct thermal performance curves, including development rate, metabolic rate, and tolerance to heat, with some studies finding thermal tolerance varying predictably with the population's thermal environment of origin, and others not [17, 88]. While we cannot exclude that survival of adults could be affected by different rearing methods across laboratories/studies, as observed with adult feeding regime (sugar fed vs blood fed, Fig. 7), it would be interesting to analyse the longevity of sugar fed *Culex* populations from other northern

areas to determine whether they also live longer at cooler temperatures.

Analysing the life traits of mosquitoes is key to evaluate the efficiency of MBD transmission, *i.e.*, vectorial capacity. Vectorial capacity is specific to the host/pathogen combination, and is influenced by vector competence, which is the ability of a mosquito to be infected and transmit a pathogen, as well as other parameters including the pathogen extrinsic incubation period (EIP), the biting rate, vector density, and the probability of vector survival [16]. Our findings suggest that global warming in Scotland will likely impact the global population dynamic of native *Cx. p. pipiens* and will help to assess the risk of *Culex* population expansion under different scenarios of climatic change. Indeed, predictions of higher temperatures and precipitations in response to climate change will likely change mosquito population dynamics and phenology, which may in turn affect arbovirus transmission potential [89–91]. Survival analyses revealed that our colony exhibited the highest longevity compared to previously described colonies at each temperature, with an unprecedented 6-month lifespan in adult females maintained at 18 °C, the current average temperature during summer in Scotland. This critical life trait impacts the vector density and the probability of daily survival, and ultimately vectorial capacity. The vector competence and the EIP of *Cx. pipiens* s.l., as well as other mosquito species, have been shown to be temperature-dependant, with lower temperatures often leading to slower virus progression, a lower number of infected mosquitoes and/or a longer EIP [92]. However, the exceptional longevity of Scottish *Cx. pipiens* females may allow for transmission even if the viral EIP is long. Conversely, the substantial reduction in their survival observed at higher temperatures could offset the increase in vector competence/shortened EIP if the mosquito lifespan approaches or falls below the duration of the EIP. It is also possible that increase in temperatures experienced by mosquitoes will result in their adaptation and shift of their thermal tolerance to warmer temperatures, as evidenced in *Ae. aegypti* [20], with lines maintained under elevated temperatures for few generations showing greater thermal tolerance.

Accurate transmission models require integrating the complex and dynamic interactions between environmental factors and mosquito life-history traits, which can influence each other across time and result in delayed downstream effects on transmission efficiency [93, 94]. Such variations can influence not only the individual mosquito, but also population-level traits across generations influencing mosquito population dynamics and disease transmission in ways that may not be immediately apparent. For example, as observed in our lifespan data, wing length measurements, used as a proxy for mosquito size, showed an inverted relationship with temperature, with larger females at lower temperatures. Mosquito size, including in *Culex* species, can affect development, longevity, reproductive output, and ultimately vectorial capacity of the mosquito population [30, 36, 58]. For instance, female body size in insects positively correlates with fecundity by increasing the number of ovarioles, suggesting that lower developmental temperatures may result in more offspring [95]. Therefore, the temperature experienced by females during development can influence the abundance of the next mosquito generation, and consequently, disease transmission potential. Moreover, several studies have shown that mosquito body size can also influences positively or negatively vector competence in *Aedes* sp. [96–101], though no significant relationship between size and vector competence was observed in *Culex* sp. has been yet described [102, 103]. These interactions governing MBD transmission dynamics are likely even more complex in long-lived mosquitoes, such as Scottish *Cx. pipiens*, which can experience multiple environmental changes-such as temperature variation-across their lifespan.

The UK has so far avoided the MBD burden seen in continental Europe thanks to a cooler climate and insular geography, which have historically acted as natural barriers against mosquito invasive species and MBD transmission by native species. However, the UK faces an increasing risk of MBD emergence, with recent detections of WNV in local mosquitoes, the widespread presence of *Culex* vectors, and warming climates that enhance mosquito activity and transmission potential. The likely establishment of Usutu virus in England further underscores how climate change is creating more favourable conditions for mosquito-borne diseases, prompting strengthened national surveillance efforts. Due to its northern location, Scotland has been largely overlooked in UK-wide mosquito surveillance, which has primarily focused on southern England and Wales [14]. While Scotland's climate remains relatively inhospitable to invasive species colonisation, native species well-adapted to temperate climates could act as vectors. Moreover, Scotland's extensive wetland habitats, providing attractive environments for both resident and migratory birds, and shifting environmental conditions are heightening the risk of zoonotic virus emergence. The establishment of a colony offers a valuable platform to measure how environmental variation affects multiple life-history traits both directly and indirectly, and model how climate change may influence population dynamics and transmission potential. Our study examined the temperature-driven phenotypic variation of Scottish *Cx. p. pipiens* mosquitoes across a temperature gradient,

providing essential life-traits data for assessing current and future risks of MBD transmission in Scotland. These findings help address a critical technical and knowledge gap, contributing to improved risk assessment and preparedness for MBD emergence in the UK.

Understanding the current thermal adaptations of mosquitoes, particularly across multiple populations spanning diverse climates and latitudes, is crucial for improving predictions of how mosquito-borne disease dynamics will respond to climate change. Simplified thermal performance models derived from only a few populations risk overlooking important variation in thermal tolerance among populations, potentially leading to inaccurate forecasts of vector-borne disease transmission under future temperature scenarios. Our study contributes to refining this understanding by providing novel data on thermal tolerance and life-history traits from a northern *Cx. pipiens* population, highlighting the importance of incorporating geographic and climatic diversity into models of mosquito thermal ecology and disease risk.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13071-026-07562-3.

Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Supplementary Material 4.

Acknowledgements
We acknowledge Sai Kulkarni for assistance in the insectaries and Thomas Ant for sharing Wolbachia FISH protocols. We are also grateful to Estela Gonzalez Fernandez for her advice on breeding *Culex* mosquitoes. We thank Colin Malcolm for helpful discussion and sharing some mosquito specimens. We acknowledge Francesco Baldini for sharing control DNA for mosquito species identification. We are grateful to all members of the Mosquito Scotland project team for their insightful discussions.

Author contributions
J-P.P.: Conceptualisation, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualisation, Writing – original draft, Writing – review & editing. D.B.: Investigation, Formal Analysis, Visualisation, Writing – original draft, Writing – review & editing. D.K.: Investigation, Project administration, Writing – review & editing. B.P.: Investigation, Formal Analysis, Writing – review & editing. R.C.: Investigation, Formal Analysis, Writing – review & editing. G.K.: Investigation, Writing – review & editing. R.B.: Investigation, Writing – review & editing. S.W.: Funding acquisition, Supervision, Writing – original draft, Writing – review & editing. H.M. F.: Conceptualisation, Funding acquisition, Supervision, Writing – review & editing. E.P.: Conceptualisation, Data curation, Formal Analysis, Funding acquisition, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

Funding
This study was funded by the UKRI Defra grant BB/X018113/1 to HF and EP and by the UK Medical Research Council (MC_UU_00034/8 to EP).

Data availability
Source data are provided with this paper and are available in the University of Glasgow repository https://doi.org/10.5525/gla.researchdata.2003. Wolbachia sequence has been deposited to NCBI, accession number PV629407.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 6 May 2026 Accepted: 30 June 2026
Published online: 31 July 2026

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