Evolution of divergent daily temporal niches shaped by male-male competition can generate sympatric speciation

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Data availability

The data underlying this article are available in the Dryad Digital Repository, at : http://datadryad.org/share/20xSLjxYGxlkU1QV-vTMITztq4XeQ427NGphEm-mNKg.

Author contributions

Titouan BOUINIER conceived the experiments, collected the data, and wrote the original draft.

Arthur BRUNAUD developed the first versions of the code. Titouan BOUINIER, Violaine LLAURENS and Charline SMADI reviewed and edited the writing at all stages of revision.

Funding

This study was funded by the European Union (ERC-2022-COG - OUTOFTHEBLUE - 101088089). Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or the European Research Council. Neither the European Union nor the granting authority can be held responsible for them.

Conflict of interest

None.

Acknowledgments

We thanks Amaury LAMBERT and Guillaume ACHAZ for their thoughtful contributions to the code and interesting questions.

Keywords: Speciation, Competition, Evolutionary Ecology, Models/Simulations

Abstract

Specialisation into different ecological niches can participate to species divergence in sympatry. Here, we focus on the partitioning into different timings of reproductive activities during the day, as observed in many insect species living in sympatry. We thus investigate the evolution of the reproductive activity timing of individuals within populations, using a comprehensive stochastic model. We used a multi-locus model to investigate the evolution of (1) the timing of reproductive activity as a quantitative trait and (2) neutral loci that may generate genetic incompatibilities among divergent individuals. We specifically explore how male-male competition for female access can generate negative frequency-dependence on the timing of reproductive activities and fuel population divergence. Our simulations in finite populations highlight the strong effect of male-male competition and operational sex-ratio on the evolution of divergent temporal niches. They also show how genetic incompatibilities fuel the differentiation among populations with divergent temporal niches, but may impair their co-existence. Our model therefore highlights male-male competition as an important factor shaping the evolution of diel niches, that may fuel sympatric speciation.

Introduction

The coexistence of multiple species in sympatry is usually thought to be linked to their partition into multiple ecological niches (J. M. Smith, 1966). Whether the ecological niches in sympatric species evolved in response to local interactions between species, or whether species diversification in sympatry results from specialization in different niches is a key question in community ecology. For instance, the competition for resources between species can fuel the evolution of species into divergent niches within the same environment (Soliva and Widmer, 1999), with specialization into different resources (Kitajima and Poorter, 2008). Within communities, species can also be partitioned into different temporal niches, with variations in breeding season (e.g. in birds, Karr, 1976) or timing of daily activities (e.g. in tropical beetles, De Oliveira Ribeiro et al., 2022 or Lepidoptera, Beck and Linsenmair, 2006). Plants flowering time is also a common example of differentiated reproductive timings, with strong genetic differentiation between closely-related, sympatric species, as for instance in *Howea* palms (Savolainen et al., 2006), or *Gymnadenia* orchids (Soliva and Widmer, 1999). Temporal niches can be shaped by abiotic factors such as temperature, but also by interactions with closely-related species (see Taylor and Friesen, 2017 for a review). In particular, reproductive interference between closely related species might favor the evolution of divergent phenology in sympatry (Devries et al., 2008). In turn, temporal segregation would also contribute to the speciation process itself, because of preferential reproduction among individuals sexually active at the same period of time (Devaux and Lande, 2009). Nevertheless, the conditions facilitating allochronic speciation (Hendry and Day, 2005) remain largely unexplored. Interestingly, temporal partitioning of species activities throughout the day has been repeatedly reported in tropical insects (see for example Caveney et al., 1995; De Oliveira Ribeiro et al., 2022; Devries et al., 2008; Lamarre et al., 2015), but the evolutionary factors shaping this temporal structure are still largely unknown.

Selection promoting divergence in the timing of reproductive activities can stem from competition for accessing mating partners. In species with separated sexes, male-male competition can be strong, especially when the adult sex-ratio is unbalanced or when there is an asymmetry in the frequency of mating between males and females (Arak, 1983; Sullivan-Beckers and Cocroft, 2010). Competition for accessing available females may impact the evolution of males timing of activities: the evolution of protandry promoted by selection linked to limited female availability has indeed been documented in a number of plant (Forrest, 2014; Zhao et al., 2020) and animal species (Morbey and Ydenberg, 2001). Furthermore, when female availability is a limiting factor for male reproduction, intense competitive interactions may occur among males and the evolution of non-overlapping timing of courtship may then reduce the deleterious effects of male-male competition. Nonetheless, whether temporal segregation could be an evolutionary response to competition is still not clear (Ekrem et al., 2025). Aggressive behaviors between patrolling males, observed both within and between sympatric species of Morpho butterflies, may have contributed to the documented divergent timing of male patrolling behavior between sympatric species (Le Roy et al., 2021). Antagonistic male-male interactions might thus contribute to the evolution of divergence in temporal niches: competitive interactions among males may generate negative frequency dependent selection on the timing of reproductive activities (Doebeli, 1996). In many insect species, reproductive activities such as pheromone release (*e.g.* in Lepidoptera, Hall et al., 1987; Shorey, 1973), patrolling for territory defense (*e.g.* in dragonflies, in Alcock, 1989), or lekking/swarming (*e.g.* in fruit flies, Shelly and Whittier, 1997) can be limited to a very short period of the day (Lamarre et al., 2015), potentially limiting interactions with heterospecific individuals. Competition for accessing suitable mating partners can therefore be a powerful driver for divergent evolution of timing of activities linked to reproduction, and might contribute to the speciation process.

The genetic basis underlying variation in the timing of reproductive activities among incipient species has scarcely been investigated. Nevertheless, in *Drosophila*, the circadian genes *period* and *timeless* were shown to control the daily mating hours in two closely related species (*D. simulans* and *D. melanogaster*), both observed in sympatry and exhibiting divergent timing of sexual activities (Sakai and Ishida, 2001). The divergence in circadian clock between these *Drosophila* species was thus suggested to enhance reproductive isolation in sympatry. Similar mechanisms were also reported in the mouse *Acomys cahirinus*, where the regulation of genes affecting the circadian rhythm is found to impact the divergence in the timing of daily reproductive activities between populations (Wang et al., 2022). The genetic basis of activity timing has been demonstrated in several species, particularly in birds (Evans et al., 2020; Hau et al., 2017), with circadian traits showing high heritability (*e.g.* in *Parus major*, Helm and Visser, 2010). An important body of research on timing of moth pheromone release demonstrates how reproductive activity can be also restricted to specific time windows (Bento et al., 2001; Cardé et al., 1996; Heath et al., 1991), notably in *Spodoptera frugiperda* (Hänniger et al., 2017; Pashley et al., 1992; Schöfl et al., 2009). Importantly, the timing of pheromone release and response was shown to be genetically controlled (Rafaeli, 2002). Polygenic control of diurnal timing of emergence was also reported in marine midges *Clunio marinus* (Kaiser et al., 2011). Notably, this emergence timing might also tune the timing of reproductive activities: since females are virgin at emergence, the timing of emergence generates a sudden burst of females available for mating and might therefore impact the timing of courtship in males. The variability and heritability of circadian activities therefore raise the possibility of an evolution of reproductive activities timing that may generate divergent evolution of temporal niches in sympatric species. Disentangling whether temporal segregation is the result of a reinforcement process caused by the poor hybrid fitness in cases of secondary contact between species, or whether such segregation is the initial driver of speciation is then an important question.

Allochrony is shown to be an important factor fuelling population divergence, even when the initial trigger of divergence stems from another ecological factor (*e.g.* host shift, Tabuchi and Amano, 2003), suggesting an effect of allochrony in the reinforcement process.

Pre-zygotic isolation caused by allochronic migrations has also been suggested to reinforce genetic divergence between populations of the butterfly *Danaus chrysippus* (D. A. S. Smith et al., 2010).

The level of post-zygotic isolation between sympatric species with divergent temporal niches is frequently unknown, but the accumulation of Bateson-Dobzhansky-Muller (*BDM* hereafter) incompatibilities (Orr and Turelli, 2001) is likely to interact with the preferential reproduction between synchronously active individuals, therefore facilitating the emergence of reproductive barriers between asynchronous populations. Such interactions between pre and post-zygotic barriers have been shown to fuel speciation by reinforcement in a model of neutral speciation triggered by isolation by distance (Baptestini et al., 2013). How much the interaction between genomic incompatibilities and temporal isolation fuels speciation is still an open question.

Here, we built a general model to investigate the effect of male-male competition on the evolution of the temporal window of daily reproductive activities and its impact on the population divergence and speciation. We assumed a single haploid species, with two separate sexes, obligatory sexual reproduction and asymmetry in the frequency of mating between males and females. We model a quantitative trait of timing of sexual activity controlled by a multilocus architecture and followed the evolution of this trait depending on the intensity of male-male competition. We also investigate the interaction between the evolution of this timing of activity and genetic incompatibilities by simulating the evolution of biallelic neutral haploid loci within the genome. These loci either (1) had no impact on reproductive success or (2) generated incompatibilities preventing reproduction between genetically divergent individuals. Using an individual-based model, stochastic simulations were conducted to identify the evolutionary mechanisms promoting the emergence

of divergent populations with segregated timings of reproductive activities in sympatry.

Methods

Model overview

We built a stochastic, individual-based model of birth-and-death processes (*e.g.* Méléard, 2016; Otto and Day, 2011), to study the evolution of daily periods of reproductive activity within species, and test for the divergent evolution of allochronic populations, depending on the presence/absence of genetic incompatibilities acting as post-zygotic barriers. Within each individually-centered simulation, each individual of the population has a set of traits (see Table 1), impacting its survival and reproductive success during the simulation.

The model, summarized in fig. 1, accurately fits to the life cycle of Lepidoptera, where immature caterpillar stages and sexually mature adult stage are strictly separated by the pupal stage. It can nevertheless be generalized to hemimetabolous and holometabolous insects (insects with multiple stages of development), as they generally display life cycles with stereotypical timing of transitions between developmental stages. We considered a population where the adult emerges from the pupa only within a restricted period of the day, so that the emergence timing generates a burst of virgin females (and males) at a fixed given period within each day, centered around a time e. We then assume that the timing of reproductive activity, expressed in both males and females, is a heritable trait named h_a, controlled by a set of B haploid and unlinked loci. Alleles interacted additively and had equal phenotypic effect sizes $\pm\frac{1}{B}$, chosen such that h_a values could range from 0 to 1. We assumed free recombination among these loci, as well as mutations occurring at a rate μ. We then used individually-centred numerical simulations to track down the evolution of the

distribution of the h_a trait within the population.

The model considered each day as a continuous time period from sunrise ($t = 0$) to sunset ($t = 1$), where an adult individual i is active only during its activity window of width $2 \cdot w_a$ (with w_a = 0.15), starting at time $t_1 = h_{a,i} - w_a$ and finishing at time $t_2 = h_{a,i} + w_a$ (bounded to $t_1 \geq 0$ and $t_2 \leq 1$). Since reproductive activities can only happen during the day, individuals with h_a values close to dawn and dusk thus have a shorter reproductive window. Individuals can mate with individuals of the opposite sex only if they happen to be active at the same time. Mated females will then generate offsprings at the start of the next day. New individuals will emerge and become sexually active on days directly following the mating day. Each day, new adults can thus join the population after they emerge from their pupae, at a time drawn in a Normal distribution of mean e, and variance v_e (similarly bounded to a $[0,1]$ interval). If an individual sexual activity period is earlier than its emergence timing, it can start mating only the day after its emergence day, within its reproductive activity window determined by h_a. Note that adults may survive several consecutive days, so that multiple generations of individuals can overlap temporally.

The simulation process used here follows the Doob-Gillespie method (see Gillespie, 1977; Tran, 2006), that allows to simplify the way individual behaviors, whose probability depends on the individual attributes (Table 1), on the parameters of the model (Table 2) and on the population state, actually happen during the course of our stochastic simulations. Here, we only described the probability of certain behaviors to happen to the different types of individuals for more clarity, but the details of the simulation process are provided in the Supplementary Materials and Methods.

Individual events

The behavior of each individual within the simulation depends on its attributes (see Table 1), that modulate its probability of survival and reproductive success at each time step.

Sex-dependent death rate

All individuals have a baseline daily death rate d during their adulthood, corresponding to death caused by factors like predation or senescence. During the reproductive period, individuals actively search for a partner, and thus are more likely to get attacked by predators, resulting in an increased death probability, modelled by a daily activity cost (named δ_e) that depends on the activity duration and applied to both males and females. Because males also engage in behavioral, competitive interactions against each other, we assume they suffer from a supplementary cost that depends on the number of other males active within the same time window $N_a^{♂}$, increasing their death rate during their activity period, with a rate $\delta_c N_a^{♂}$. Egg-laying is also likely to generate a daily metabolic cost to females (named δ_l): we thus assumed that the more eggs a female has laid, the more likely she is to die.

Mating success

This model assumes differences in reproductive traits between males and females: males can mate indefinitely due to their ability to produce smaller and more quickly replenished gametes, while females have a limited number p of mating opportunities, due to a more costly production of gametes. They nevertheless stay fully receptive until this number is reached, and may produce offspring after the first mating event. The availability of females thus becomes the limiting factor for male reproduction, generating intense male-male competition.

We assume that active males and active receptive females probability of mating depends on the current number of active individuals of the opposite sex, as well as an encountering rate β (see Table 2). Here we assume no spatial structure, so that there is equal encountering probability for all individuals being active at the same time. The mating rate is thus simply represented by $\beta \cdot N_a^{♂} \cdot N_a^{♀}$.

Each day, we assume that each mated female generates a number of offspring which depends on population density. This number depends on the parameter b (the maximum number of offspring per female per day), on the carrying capacity of the environment (K) (see Table 2), and on the number of mated females alive at the start of the day ($N_m^{♀}$). As the number of mated females increases, each female produces fewer viable offspring, because of competition for resources. This reflects the limited availability of resources for the offspring, leading to higher mortality rates when too many offspring are born. Notably, since mated females incur a δ_l cost proportional to the number of eggs laid, in a high density population, females will have a longer life expectancy since they lay less eggs.

The number of offspring per female is drawn individually from a Poisson distribution of parameter λ_p. This parameter is computed using the following formula:

$$\lambda_p = b\left(1 - \frac{N_m^{♀}}{K}\right). \tag{1}$$

The number of mated females $N_m^{♀}$ is always inferior to the carrying capacity K in our simulations. We thus never encounter the case where λ_p is negative. Once a female has mated at least once, it can generate offspring every day after the mating day, until its death. After completing p reproductive events, females transition from being receptive to unreceptive for mating for the rest of their life.

Offspring generation

To investigate the impact of post-zygotic incompatibilities on the divergent evolution of allochronic populations, we also assume B biallelic, unlinked, haploid loci that may influence the survival of the offspring produced by different parental pairs, but otherwise not influencing individual survival. In each of these haploid B loci, two alleles (0 or 1) can occur, and the viability of the offspring of a parental pair depends on the genetic distances across these B loci between the parents. The genetic

distance was calculated using the Hamming distance (Baptestini et al., 2013), where the distance between the genomes of any two individuals, x and y in the population is given by the following equation:

$$D_H = \frac{1}{B} \cdot \sum_{i=1}^{B} |x_i - y_i| \tag{2}$$

with x_i and y_i the allele values at the locus i for the individuals x and y respectively.

Note that $0 \leq D_H \leq 1$, and $D_H = 1$ is the maximum genetic difference between two individuals. For simplicity, we set a threshold value G above which mating between individuals with $D_H > G$ does not generate any viable offspring. While this threshold is probably more drastic than the actual effect of incompatibilities actually arising in natural populations, this simplification does allow to relevantly implement reproductive barrier among genetically divergent individuals, and has been used in previous models (see Yamaguchi and Iwasa, 2017 and de Aguiar et al., 2009 for instance).

If the offspring does survive, it eventually emerges as adult, at a time drawn in a truncated Normal distribution of parameters (e, v_e) restricted to $[0, 1]$. The sex of the individual is randomly chosen, assuming a balanced sex-ratio at birth. We assumed free recombination between loci, and a mutation rate of μ per loci (identical for neutral and activity timing loci), flipping its value from 0 to 1 or *vice-versa*. In cases where the mother has mated with multiple males (in simulations with $p > 1$), one of them will be chosen uniformly at random to sire the offspring.

Summary statistics drawn from simulation outcomes

To detect whether differentiated populations evolved during the course of a simulation, we track down the distribution of the trait h_a in the simulated populations and aim at detecting the num-

ber of peaks in this distribution. In Python (3.11.7), we used the *find_peaks* function from the "scipy.signal" package (Virtanen et al., 2020), to detect peaks and identify their position to define sub-populations. A group of individuals with similar timing of activities that diverge from the ancestral population is then considered as an actual differentiated sub-population only when (1) it lasted more than forty days within the simulation and when (2) the position of the peak within the differentiated sub-population did not vary by more than 0.15 units of time over a day. Those conservative requirements were determined from previous simulations, when searching for parameters that would guarantee large enough sub-population sizes and stable sub-populations over the length of a simulation. We must note that in our simulations, we only observed cases with either one or two peaks, (i.e. either one or two differentiated sub-populations), no matter the set of parameters used.

We considered three categories of sub-populations in our model:

1. *Immediate* category, when the average reproductive activity within a sub-population was centered around the timing of emergence e, with a leniency of ± 0.15.

2. *Dawn-shifted* category, when the average reproductive activity was earlier than the timing of emergence e, meaning mating starts happening on the day after the emergence day for those individuals.

3. *Dusk-shifted* category, when the average reproductive activity was later than the timing of emergence e, meaning freshly emerged individuals could start mating on the day of their emergence.

We also characterized the shape of the distribution of reproductive activity timings within each sub-population. Using the *curve_fit* function from the "scipy.optimize" package, we fit a Gaussian curve on these distributions at the end of each simulation. When two sub-populations are detected, we separate our dataset and fit a curve to each of the two sub-populations. We then evaluate the

distribution width by using this standardized Gaussian curve, and measure the full width at half maximum (*FWHM*) to capture the shape of the curve. Additionally, when two sub-populations were present, we computed the distance between their respective h_a peak.

These metrics provide a basis for determining whether two sub-populations are distinctly differentiated or if their reproductive activity timings exhibit potential overlap.

When differentiated sub-populations were observed at the end of a simulation, we quantified the level of genetic differentiation between the two sub-populations, using the F_{st} values computed from genetic variations observed at the B neutral loci. We used the minima between the two peaks in the reproductive activity distribution curves to determine to which sub-population each individual belonged to. Individuals with h_a values greater or lesser than this minima are sorted to either sub-population. The F_{st} is then calculated according to the following equation, from Hudson et al., 1992:

$$F_{st} = \frac{\pi_{inter} - \pi_{intra}}{\pi_{inter}} \tag{3}$$

With π_{intra} and π_{inter} the average number of pairwise differences between two individuals of the same/different sub-population respectively

Note that under the assumption that intra-population variations are always smaller that inter-population variations, $0 \leq F_{st} \leq 1$, $F_{st} = 1$ is the maximum level of genetic differentiation between the two sub-populations. When differentiated sub-populations were observed at the end of a simulation, we also measured the level of linkage disequilibrium (*LD*) among loci of the neutral genome, among the loci controlling reproductive activity timing h_a, as well as between the two types of loci. We computed the coefficient of linkage disequilibrium D, where, for an allele A of a loci and an allele B of a different loci, both of respective frequency p_A and p_B, $D = |p_{AB} - p_A p_B|$. Departure of D from zero indicates significant *LD* between loci.

Numerical simulations and equilibria

We ran each simulation for $T = 500$ time units for each repetition, ensuring that the equilibrium is reached for simulations, and used a range of reasonable parameters to ensure the emergence and co-existence of differentiated sub-populations. Parameters and attribute range values are provided in Tables 1 and 2. All simulations ended after 500 days, or when the entire population became extinct. The initial conditions for all simulations are a population of hundred individuals with a balanced sex-ratio, where all females are initially virgin. The initial genotypes at the loci controlling h_a of all individuals are set to a identical assortment of 0 and 1, such that their initial phenotypic value is 0.5. The neutral loci are initialized with values all equal to 0. A hundred replicates were run for each condition and the range of parameters used is given in Table 2. Python (3.11.7) was used for modeling purposes and data cleaning, using the packages ”random”, ”numpy”, ”scipy” and "seaborn". R (4.4.0) was used to plot all results, using the "ggplot2" package.

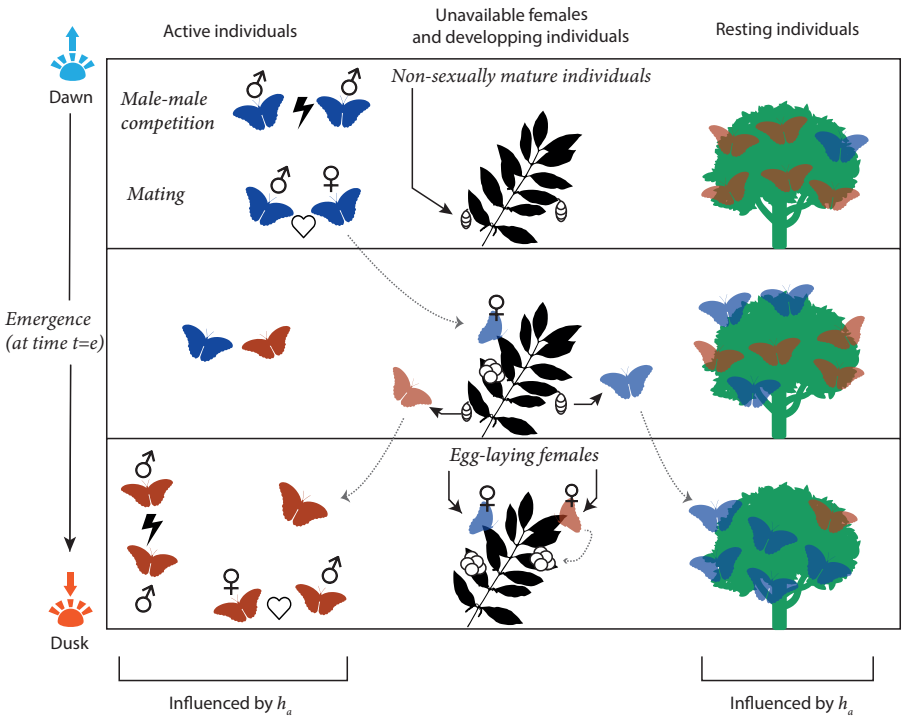

Figure 1: **Schematic view of the model describing variations in activity patterns in a butterfly population with two main sub-populations, with individuals with early and late adult reproductive activity times represented by blue and orange butterflies respectively.** The scheme describes a typical day in our simulation, where two sub-populations have successfully differentiated. Individuals active at the same time can participate to male-male competition, or mate with individuals of the opposite sex. Once mated, females become unreceptive for reproduction and lay eggs that will give rise to new individuals. These non-sexually mature individuals (represented as pupae on the scheme) will become mature adults over the next few days. At the same time $t = e$ each day, some sexually mature adults can emerge from the previously produced pupae. If an individual activity timing is later than the emergence timing, it will be become active within the same day, potentially mating right after emergence. In contrast, individuals emerging after their activity timings will start mating only the following day.

Results

Evolution of sub-populations with earlier reproductive activity

Assuming that females are able to mate at maximum once throughout their life ($p = 1$), and assuming no genetic incompatibilities, our simulations result in either a unimodal or a bimodal dis-

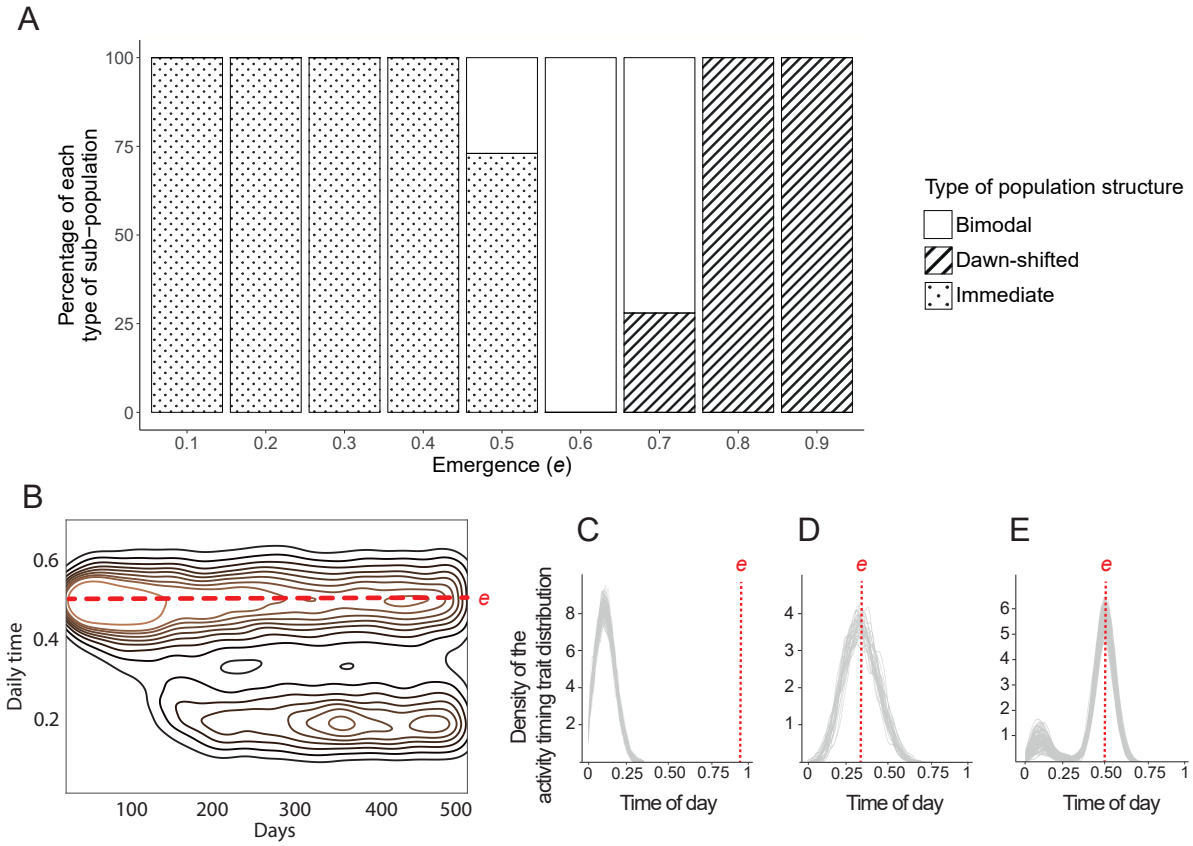

Figure 2: **Emergence of sub-populations with different temporal niches depending on the timing of emergence of adults.** (A) Percentage of simulations where either one or two sub-populations were observed after 500 days, for 100 replicates per value of the timing of adult emergence e. (B) presents the evolution of the activity timing trait throughout a simulation where bimodality emerges. (C), (D) and (E) are distributions outcomes of simulations for each type of distribution. Two types of unimodal distribution of the timing of reproductive activity can be observed after 500 days: (C) a single *dawn-shifted* population, where the average time of reproductive activity is below the emergence time e or (D) a single *immediate* population. Bimodal distribution of timing of activities leading to the co-existence of two sub-populations can also be observed (E), with both *dawn-shifted* and *immediate* sub-populations. The red dotted lines in (B), (C), (D) and (E) show the emergence timing in our examples ($e = 0.5, 0.9, 0.3$ and 0.5 respectively). All simulations were run assuming the same values in the other parameters : $\beta = 3, \delta_c = 0.1, G = 1, v_e = 0.05, p = 1, K = 1000$.

tribution of the timing of reproductive activities, depending on the timing of emergence of adults e assumed (fig. 2).

- When a unimodal distribution of activity was observed, the average timing of reproductive activity usually follows the timing of emergence for most e values (fig. S1A), and a single *immediate* population was thus observed. When the emergence occurs late during the day ($e \geq 0.7$), individuals are not active close to the emergence time happening at dusk, but rather at dawn (*i.e.* single *dawn-shifted* population). Since our model assumes no mating during the night, late reproductive periods are probably counter-selected. Late timing of adult emergence may thus favor the evolution of reproductive activities early in the morning.

- When a bimodal distribution in the timing of activity is observed, two sub-populations with different temporal niches (one with a *dawn-shifted* niche and one with an *immediate* niche) can co-exist. The sub-population with the *dawn-shifted* niche has an average timing of reproductive activity close to the start of the day, while the sub-population with an *immediate* niche displays an average timing of activity close to the moment of adult emergence, where more receptive females are available (fig. S1B).

Characterisation of the distributions of the reproductive activity time h_a revealed that when assuming no genetic incompatibilities, the average distance between peaks (when in a bimodal case) in a day was around 0.3 time unit (fig. S2A) while the average peak width ranged from 0.16 to 0.19 time unit (fig. S2B). Our differentiated subpopulations thus likely have limited temporal niche overlap.

Interestingly, when the emergence of adults was assumed to occur either very early or very late during the day, the proportion of simulations where a bimodal distribution is observed decreases (fig. 2). Nevertheless, the outcomes of simulations assuming very early *vs.* very late emergence time differ. When emergence is very early, reproductive activity can start occurring within the

same day, and selection thus favors the evolution of *immediate* temporal niches, allowing males to mate with virgin females immediately. The lack of available time earlier than the emergence time likely prevents the evolution of a second population with even earlier timing of activity. In contrast, when the emergence occurs very late in the day, we observed the evolution of a single *dawn-shifted* population. Since males with *immediate* timing of reproductive activity would have little mating opportunity before dusk, individuals that are active the earliest on the following day become advantaged, because they are more likely to encounter the virgin females emerged the previous evening. Overall, these results suggest that the evolution of temporally differentiated reproductive sub-populations is heavily influenced by the timing of emergence (e). Specifically, emergence occurring in the middle of the day allows for temporal niche differentiation, as there is more available temporal space. On the contrary, extreme emergence times (either very early or very late) restrict reproductive timing variability, preventing the evolution of differentiated temporal niches.

We subsequently tested the effect of the variability in the timing of adult emergence (v_e) throughout the day, as it can impact the probability of encounter between males and newly emerged receptive females. Emergence of differentiated sub-population was favored by intermediate values of v_e (fig. S3), while low and high v_e values resulted in unimodal and dawn shifted populations respectively. See Appendix A for a more in depth analysis of the effect of v_e. Variation in the timing of adult emergence within a population thus plays a key role in shaping the evolution of temporal niches. While some variability is essential for individuals to diverge in their activity patterns from the emergence peak timing, completely uniform emergence within a single day prevents the development of distinct temporal niches.

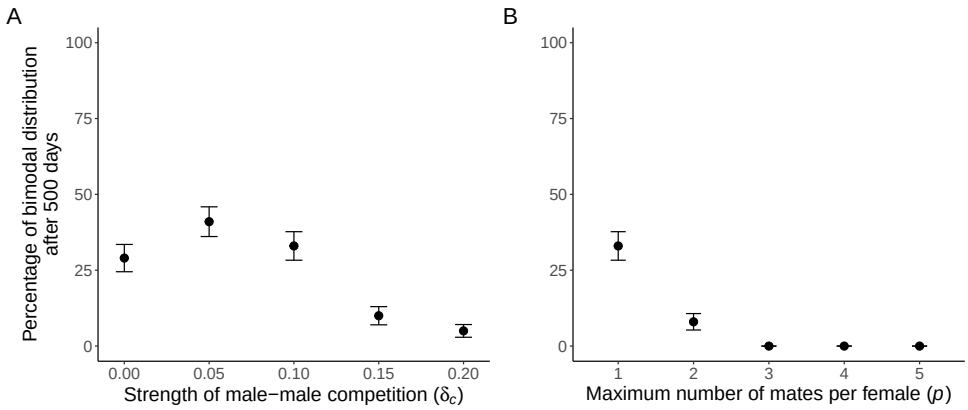

Figure 3: **Effects of the male-male competition and male-female asymmetry on the evolution of sub-populations with different temporal niches.** (A) Effect of the intensity of male-male competition (δ_e) on the percentage of simulations where a bimodal distribution was observed after 500 days, for 100 replicates per condition. Intermediate values (around $\delta_c = 0.05$) lead to the higher percentage of simulations with coexistence of *dawn-shifted* and *immediate* sub-populations. (B) Effect of maximum mating events per female on the percentage of simulations where a bimodal distribution was observed after 500 days for 100 replicates per condition.
Error bars show the standard deviation (SD) computed over the 100 replicates per value of each parameter. All simulations were run assuming the same values in the other parameters $\delta_c = 0.1$, except in 3A, $\beta = 3, G = 1, e = 0.5, v_e = 0.05, p = 1$, except in 3B, $K = 1000$.

Behavioral male-male competition and competition for receptive females promoting the evolution of differentiated sub-populations

To test how much the density-dependent male-male competition generates negative frequency dependent selection on the timing of male sexual activity, we compared the proportion of simulations where a bimodal distribution of the timing of activities was observed at $N = 500$ days, when assuming different levels for the cost of behavioral male-male competition. To test for the effect of operational sex-ratio on negative density-dependent selection, we also compare simulations allowing either a maximum of one mating event per female ($p = 1$) *vs.* multiple mating events per female (from $p = 2$ to $p = 5$), using $\delta_c = 0.1$ (fig. 3B). We indeed observe the significant effect of asymmetry in mating opportunity between males and females in the evolution of differentiated

temporal niches: when the maximum number of partners possible for a female increases, the proportion of simulations with co-existing differentiated sub-populations drastically decreases, from one third of simulation $p = 1$, to being never observed when $p \geq 3$. The mating asymmetry alone is thus sufficient to generate negative frequency-dependent selection on the timing of reproductive activities through male-male competition. We hypothesize that this competition is driven by a limited availability of females for mating, inherently creating male-male competition for access to females. This competition, in turn, fosters negative frequency-dependent selection, favoring reproductive activity at times when fewer males are competing simultaneously. Female mating limitations thus strongly favors the emergence of bimodal distributions in the reproductive activity timing of individuals.

When testing how much behavioral male-male competition affects temporal differentiation, we nonetheless observe an increase in the probability of differentiation into two sub-populations when low competition exists ($0.05 \geq \delta_c \geq 0.1$), compared to simulation without competition. However, when the competition becomes too intense ($\delta_c > 0.1$), the probability of observing differentiated populations decreases, reaching lower values than in simulations without competition. Such decrease may be explained by competitive exclusion between the two sub-populations (fig. 3A): when low competition was assumed ($\delta_c = 0.05$), the duration of coexistence between sub-populations is on average higher (fig. S4B) compared to simulations without any competition ($\delta_c = 0$), but decreases as the strength of male-male competition increases. When competition gets too intense ($\delta_c > 0.1$), the duration of coexistence became lower than in simulations assuming no competition. The *dawn-shifted* sub-populations generally had a much smaller size as compared to *immediate* ones (see fig. 4B). This difference in population size increases with δ_c, meaning that *dawn-shifted* sub-populations are much more likely to undergo extinction compared to *immediate* subpopulations. This decrease in coexistence due to competitive exclusion of the *dawn-shifted* sub-populations explains the lower probability of observing differentiated sub-populations with

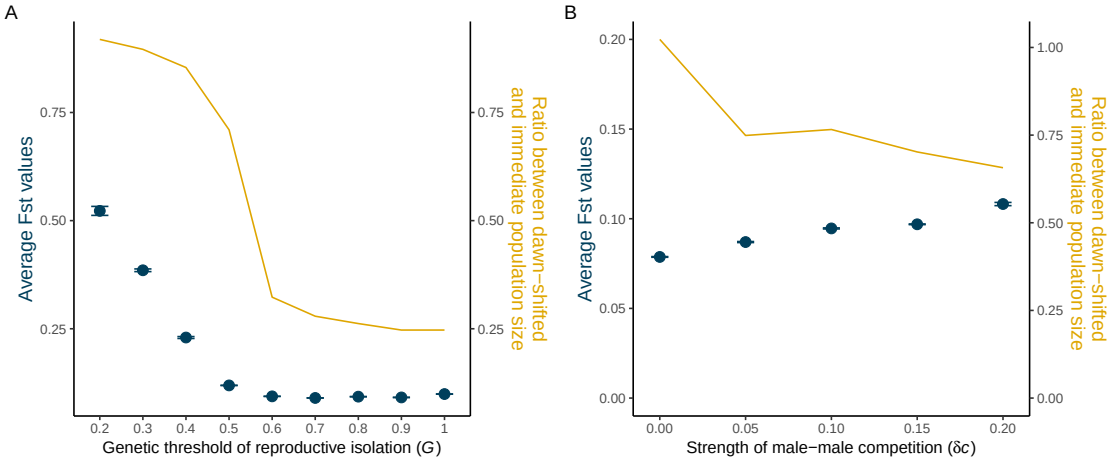

Figure 4: **Effect of genetic incompatibility and male-male competition on the genetic differentiation between sub-populations with different temporal niches.** The average F_{st} between sub-populations where a bimodal distribution was observed after 500 days, assuming genetic incompatibilities is shown, for 100 replicates per values (blue dots). The ratio between the *dawn-shifted* and *immediate* population size measured at the end of a simulation is also shown (yellow line) (A) Effect of the proportion of loci G required for mating incompatibilities on the level differentiation between sub-populations, estimated by F_{ST}. Small and moderate values of G promote high F_{st} values between sub-populations. (B) Effect of male-male competition intensity δ_c on the F_{st} values between differentiated populations. When the male-male competition intensifies, average F_{st} between sub-populations increases.
Error bars show the SD computed over the 100 replicates per value of each parameter. All simulations were run assuming the same values in the other parameters: $\beta = 3, \delta_c = 0.1, G = 0.5$ for fig. $4B, e = 0.5, v_e = 0.05, p = 1, K = 1000$.

high δ_c values.

We thus identify two forms of male-male competition influencing temporal segregation: indirect competition for access to receptive females for mating, as well as direct, density-dependent competition between males (*e.g.* behavioral interactions between males).

Genetic differentiation favored by competition and genetic incompatibilities

We then explore the effect of genetic incompatibilities in the evolution of differentiated sub-populations with divergent distributions in the timing of reproductive activities. We thus investigate

the effect of the threshold of genetic divergence at the neutral loci G, above which reproduction between pairs of individuals becomes unsuccessful. Fig. S5 shows that G has a strong effect on the probability of evolution of differentiated sub-populations with divergent temporal niches:

- When the threshold G is low, *i.e.* when few allelic variations between parents at a single locus are sufficient to prevent the generation of viable offspring, the evolution of differentiated sub-populations is relatively limited. Such drastic incompatibilities may strongly limit offspring viability and thus prevent the frequent evolution of unusual timings of reproductive activity.

- When assuming intermediate values ($0.4 \leq G \leq 0.5$), the percentage of simulations where we observe the evolution of differentiated sub-populations becomes much more likely ($>$ 98%), but decreases when the threshold further increases. Note that when $G = 1$, genetic incompatibilities are absent.

The evolution of differentiated populations is thus more likely to happen for moderate values of the threshold G, allowing sufficient successful mating, while preventing gene flown between sub-populations. The level of genetic differentiation between these sub-populations can be estimated using the fixation index (F_{st}).

When assuming genetic incompatibilities ($0 < G < 1$), the F_{st} values are higher when G is small (fig. 4A). Low values of G promote assortative mating between individuals with similar genotypes, driving the differentiation between *dawn-shifted* and *immediate* sub-populations. When the threshold G becomes even higher ($G \geq 0.6$), F_{st} values become low, coinciding with the decrease in the probability of population differentiation shown in fig. 4.

When studying the effect of the genetic incompatibilities promoting the co-existence of the two sub-populations, we found that the duration of the co-existence of the two sub-populations was maximal when $G = 0.5$ (fig. S4A). Notably, when $G > 0.5$, coexistence was much shorter, which

probably explain why fewer differentiated population are observed at the end of simulations in these cases, as they struggle to maintain themselves. In extreme cases ($G = 0.1$), every simulation lead to the extinction of the whole population, because most individuals failed to generate any offspring (fig. S5), while for values of $G > 0.2$, no extinction is observed. While the effect is weak, stricter threshold (*i.e.* lower G values), promotes a larger temporal gap in reproductive activity timing between sub-populations (fig. S2A), as well as narrower width of peaks (fig. S2B). Genetic incompatibility thus seem to reinforce the temporal divergence between the two sub-populations, as well as the specialisation into narrower temporal niches.

Furthermore, the strength of male-male competition also had an effect on genetic differentiation between populations (captured by F_{st}), with genetic differentiation increasing with the intensity of competition (fig. 4B): F_{ST} values where significantly higher in simulations with high competition ($\delta_c = 0.2$) as compared to simulations without ($\delta_c = 0$). Thus while high values of δ_c decreases the probability of population differentiation because of competitive exclusion of the *dawn-shifted* population (see fig. 3A), in the fewer cases where sub-populations do evolve (see section above), high levels of genetic differentiation are favored by the intense level of competition, counter-selecting individuals with intermediate values of h_a.

Finally, to investigate the coupling between pre and post-zygotic barriers to gene flow between the two sub-populations, we quantified linkage disequilibrium among neutral loci, activity timing loci, and between both types of loci. When computing D in the whole population, we can observe that for lower values of G, D display higher median values, but also higher maximum values (fig. 5). Interestingly, activity timing loci consistently display significantly higher D compared to neutral loci, showing the consistent genetic differentiation created by temporal segregation. Stricter genetic incompatibilities are nonetheless promoting linkage disequilibrium more frequently, as well as more strongly. This is expected, as a stricter threshold for genetic incompatibilities is likely to fix more alleles at the different loci within the two populations. Stricter genetic incompatibili-

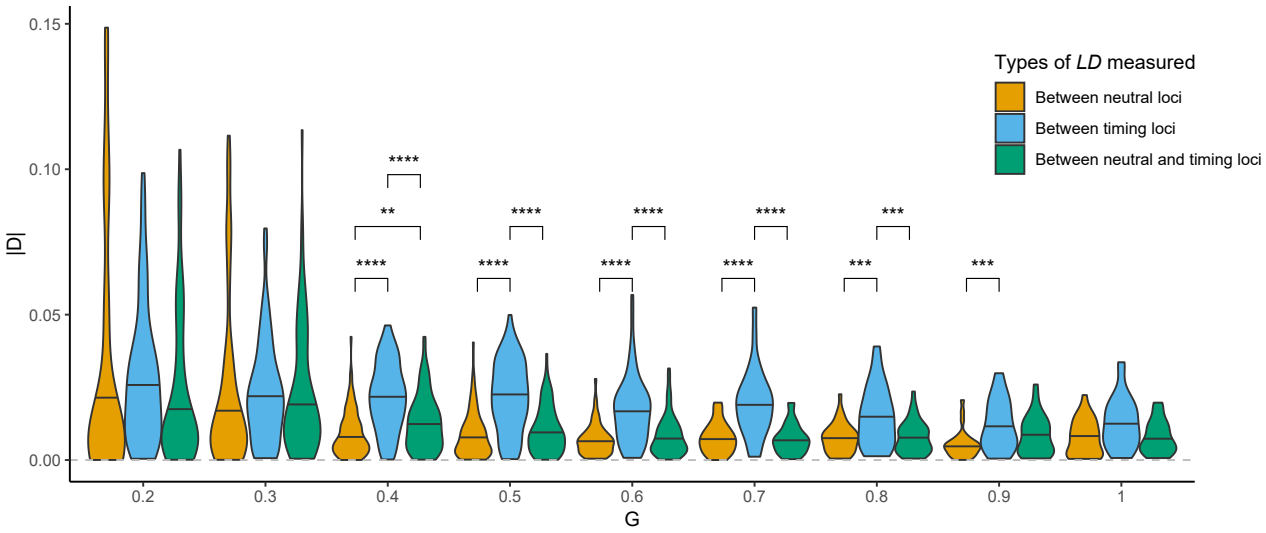

Figure 5: **Characterisation of the linkage disequilibrium between activity time and the neutral loci, by computing the |–*D* value**. We quantified linkage disequilibrium between neutral loci (yellow), activity timing loci (blue), and between both (green). Significant differences between D values are indicated. We can observe that for lower values of G, D display higher median values, but also higher maximum values. Interestingly, activity timing loci consistently display significantly higher D compared to neutral loci. Stricter genetic incompatibilities are thus promoting linkage disequilibrium more frequently, as well as more strongly, especially for activity timings loci. All simulations were run assuming the same values in the other parameters: $\delta_c = 0.1, \beta = 3, e = 0.5, v_e = 0.05, p = 1, K = 1000$.

ties are thus promoting more frequent linkage disequilibrium between loci acting as post-zygotic barrier and the locus controlling the timing of reproductive activity, acting as pre-zygotic barrier. We also observe some level of *LD* between activity timing loci and neutral loci, indicating some level of coupling between pre and post-zygotic mechanisms of reproductive isolation.

Genetic differentiation between sub-populations thus appears to be positively correlated to both the strength of behavioral male-male competition and the incompatibility threshold between divergent genomes. The linkage disequilibrium between activity timing loci and neutral loci generates a coupling of pre and post-zygotic barriers to gene flow between sub-populations that may further promote sympatric speciation.

Discussion

Evolution of temporal niches of reproductive activities

Using our model of *daily* reproductive activity niche, we show that sub-populations with different timings of reproductive activities can evolve through time. Because we assumed that offspring have a timing of reproductive activity intermediate between their parents (*modulo* the mutational effect μ), our model limits the emergence of temporal differentiation within our simulated populations. Despite this reduced opportunity of divergence stemming from the genetic architecture assumed for the control of the timing activity in our model, we nevertheless frequently observed differentiation into two subpopulations. The differentiation into different temporal niches is mainly due to the limited availability in available females, but is also promoted by costs generated by antagonistic male-male interactions. Negative density-dependent selection acting on the timing of reproductive activities can therefore promote differentiation into different temporal niches. Another model recently investigated the ecological factors promoting daily allochronic speciation in the *Spodoptera frugiperda* moth (Van Doorn et al., 2024), by assuming divergent ecological se-

lection favoring divergent adaptive timings of reproduction in the rice *vs.* corn eating populations. In line with our own results, the operational sex-ratio (controlled in their model by monogamy *vs.* polygamy during the night) is tuning the intensity of male-male competition and the resulting negative frequency-dependent selection acting on the activity timing was a key parameter shaping the evolution of allochrony.

Our model generates allochrony strictly based on male-male competition for virgin females, without assuming any other ecological mechanisms generating disruptive selection. However, when assuming independent control of the activity timing between males and females, we found that temporal segregation rarely emerged (fig. S6). This suggests that males strongly drive temporal differentiation in our model. When assuming a common control of reproductive activities in males and females, the emergence of differentiated sub-populations is likely promoted by assortative mating between individuals with similar neutral genomes (Kondrashov and Shpak, 1998), stemming a from synchronicity of reproductive activities, so that the timing of reproductive activity acts as a grouping magic trait (Kopp et al., 2018), which might be reduced when the timing of activity evolves independently in males and females, therefore restricting the conditions enabling the evolution of differentiated timing of activity (fig. S6). Moreover, we observe linkage disequilibrium between our ecological trait (activity timing) and neutral traits when assuming common control of reproductive activity between males and females. This observation aligns with Dieckmann and Doebeli (1999) prediction that *LD* between neutral and ecological traits facilitates speciation under non-random mating. We observe the direct effects of this in our model, as when we do not take into account our neutral trait into the assortative mating (*e.g.* $G = 1$), both F_{st} and the probability of temporal differentiation are greatly reduced.

In previous models describing the evolution of flowering times, negative density dependent selection stemming from pollinator availability has already been described as involved in population subdivisions into different temporal niches (Devaux and Lande, 2008). Nevertheless, this division

was generally found symmetrical around the ancestral flowering time. In our model however, the evolution of differentiated temporal niche is not symmetrical. We indeed observed an asymmetrical distribution in the *daily* model, with new sub-populations having earlier activity timings, due to competition for receptive females. We never observed *dusk-shifted* sub-populations, where individuals would be selected to be active later than emergence in a day. This shows an asymmetric positive selection for earlier h_a values and not for later ones, because of male-male competition to access virgin females as soon as possible during the day.

In perennial species where individuals are potentially available for reproduction all year round, changes towards earlier breeding times during the year is shown to occur in wild populations of *Parus major* (Charmantier et al., 2008). Furthermore, at the daily scale, fitness advantages associated with earlier sexual activity (mate guarding and extra-pair mating) in males were reported in behavioral experiments in *Parus major* (Greives et al., 2015). These empirical results suggest that changes in the timing of reproductive activities can occur in the wild, because of both plasticity and/or genetic variations, and might be favored by selective pressures. In our model, we assumed that reproductive activity times were independent from the emergent time, which was fixed during our simulations. Nevertheless, the reproductive timing of activity (modelled here as h_a) and the emergence (e) are likely co-evolving in natural populations and are probably jointly influenced by the circadian clock, as shown in Drosophila (Calatayud et al., 2007; Kumara et al., 2015). Supplementary simulations allowing the emergence timing to evolve independently of reproductive timing of activity demonstrated the emergence of differentiated sub-populations (fig. S7), both evolving towards earlier activity timings. These results demonstrate the strong effects of negative frequency dependence stemming from male-male competition on sub-population differentiation and coexistence. In this model, selection on being active as early as possible is strong in males given the absence of a fixed emergence timing during midday. The two sub-populations with earlier activity timings did not merge, even in the absence of genetic incompatibilities. This shows how negative

frequency dependence can maintain two populations in sympatry, with separated temporal niches. The evolution of the temporal window of reproductive activity predicted by our model could thus happen in natural populations, if genetic variations involved in the circadian clock controls for the timing of reproductive behavior, like courtship or mate searching activities.

Ecological factors promoting temporal divergence between sub-populations

In our model, the co-existence of sub-populations was generally transient, but varied depending on the strength of male-male competition δ_c and the threshold of genetic divergence G. We showed that moderate values of δ_c and G led to a longer duration of the co-existence for sub-populations. The limited duration of coexistence may be linked to the absence of joint evolution of other isolating mechanisms, such as host plant specialisation or coupling with abiotic factors. In our model, we indeed investigated the effect of neutral mutations triggering incompatibilities between divergent individuals. Nevertheless, if these mutations accumulating in the sub-populations with different temporal niches were under divergent selection, this may further reinforce co-existence of differentiated populations.

Temporal differentiation can be facilitated by variation in resource availability throughout the day, that may condition the timing of feeding *vs.* reproductive activities. In insects, host shifts may thus enhance the evolution of temporal niches. For instance, variation in the timing of flowering (afternoon *vs.* dusk) was reported among closely-related species of sphingophilous plants of the genus *Hemerocallis* (Hasegawa et al., 2006). Specialisation into these different plant species may induce temporal divergence in pollinators. Multiple other plants also display such daily variations flowering/pollen release patterns (Matsumoto et al., 2015; Stone et al., 1996), that may condition the evolution of daily temporal niche in pollinating insects.

Abiotic factors such as temperature also may also enhance allochronic speciation, if the reproductive success is lower when individuals are not in their optimal temperature range (Dolgin et al., 2006; Keller and Seehausen, 2012). Since thermal conditions usually vary throughout the day, the evolution of the temporal niches can be influence by variation in adaptation to thermal conditions. Differences in reproductive success was detected between sister species locally adapted to different thermal environments (Matute et al., 2009), probably as a by-product of divergent adaptation. As variations in selection of thermal resistance hinder local adaptation, species are likely to be adapted to a relatively small range of temperature conditions (Kawecki and Ebert, 2004), and variations in thermal niches can be a strong barrier to reproduction. These temperature fluctuations are shown to be a source of diversification among species (Kleckova et al., 2015), with empirical data on animal and plant species (Keller and Seehausen, 2012). The evolution of divergent thermal tolerance could co-evolve with the evolution of divergent temporal niches, at both the seasonal and daily scales.

Allochrony as a reinforcement mechanism in the divergence of species

Allochrony could also act as a reinforcement mechanism, when hybrids are already counter-selected because of post-zygotic barriers. In Butlin and Smadja, 2018, such coupling between pre and post-mating barriers was shown to evolve, but disentangling whether allochrony is the driving force of speciation or just stem from reinforcement remains challenging (see Taylor and Friesen, 2017).

To study the emergence of reproductive barriers in our population, we observed population dynamics with and without post-zygotic barriers to reproduction, represented by non-viable zygotes when the parents were genetically differentiated. When those genetic incompatibilities were

introduced, the evolution of sub-populations with different temporal niches was more likely to happen for certain values of the genetic threshold G. More importantly, genetic incompatibilities fuel genetic differentiation between sub-populations, as the gene flow between sub-population keeps occurring in absence of these genetic incompatibilities. Potential sympatric speciation could thus be driven by incompatibility selection (Artzy-Randrup and Kondrashov, 2006), even in the absence of other forces promoting ecological divergence. By testing the proportion of loci G required for incompatibilities, we found that intermediate values seemed to promote the longevity of small-sized *dawn-shifted* sub-populations. These values seem to allow a low level of gene flow that promoted co-existence, while allowing genetic differentiation between sub-populations. This is consistent with simulations for neutral speciation by distance (Baptestini et al., 2013, de Aguiar et al., 2009), showing similar results when incorporating a genetic mating distance G, with intermediate values of maximum genetic distance for reproduction producing higher counts of species. Temporal divergence could thus act as a coupled mechanism to other differentiation factors, by limiting gene flow between sub-populations.

Furthermore, during the differentiation into different temporal niches, offspring with intermediate temporal niches could suffer from elevated competition costs, as they would pay costs from negative interactions with individuals from both parental temporal niches. These intermediate offspring also have a low chance of occurring when genetic incompatibilities are added, since those two sub-populations are usually genetically differentiated, and hybrids were considered inviable. According to simulations performed by Liou and Price, 1994, having inviable hybrids can lead to higher rates of speciation than with infertile ones, because the lack of individuals with intermediate phenotypes reduces competition (for resources/mating partners) for both sub-populations. This was also shown to make extinction less likely for sub-populations, thus promoting coexistence between divergent populations. This coexistence is thus likely conditioned by the availability of empty temporal niches. Indeed, our simulations showed that a certain width of temporal space

is necessary for sub-populations to emerge earlier within the day, as they otherwise are too close to the alternative population and male-male competition may then lead to competitive exclusion. Smaller niches would result in more temporal space available for sub-populations to emerge, and thus provide more opportunities for speciation (Sexton et al., 2017). We indeed observed that temporal niches were narrower and further apart when implementing stricter threshold for genetic incompatibilities (fig. S2), a behavior akin to reinforcement. Niche breadth is generally shown to be an important factor influencing speciation (Hardy and Otto, 2014) and this effect is confirmed in our study, showing that gradual evolution of divergent temporal niches can be promoted by post-zygotic incompatibilities.

Our study therefore highlights the temporal variation in reproductive activity as an important component of the ecological niches, potentially reinforcing mating barriers between diverging sub-populations.

Data availability

The data underlying this article are available in the Dryad Digital Repository, at http://datadryad.org/share/20xSLjx vTMITztq4XeQ427NGphEm-mNKg.

Tables

Table 1: Individual attributes

Attribute	Description	Values
sex	Sex of the individual	"M" or "F"
h_a	Center of the reproductive activity period	$0 \leq h_a \leq 1$
w_a	Range of the reproductive activity period	0.15
E	Number of eggs laid by a mated female	Variable positive integer

Table 2: Parameters of the model and baseline values used in numerical simulations

Parameter	Description	Values
β	Encountering rate between active males and available females	3
d	Basal death rate	0.1
δ_e	Mate searching energetic cost for all individuals	0.1
δ_c	Competition cost in active males	$0, 0.05, ..., 0.02$
δ_l	Egg laying cost in mated females	0.1
p	Maximum number of partners per female	1,2,3,4,5
b	Mean offspring number per female per day	5
e	Emergence peak time	$0.1, 0.2, ..., 0.8, 0.9$
v_e	Variance in the timing of emergence	0.05
μ	Mutation rate of loci	0.03
B	Number of loci for each trait (neutral, activity timing)	50
G	Genetic threshold of reproductive isolation	$0.1, 0.2, ..., 0.8, 0.9, 1$
K	Carrying capacity of the environment	1000

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SupplementaryMaterial