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

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Abstract

Specialisation into different ecological niches can participate to species divergence and co-existence 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, employing a Doob-Gillespie-like simulation process. We model 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. It also shows how genetic incompatibilities fuel the differentiation among populations with divergent temporal niches, but may impair their co-existence. We then compare our results on the evolution of daily temporal niches to a previous model investigating the evolution of seasonal phenology and show the significant effect of overlapping vs. non-overlapping generations on the evolution of daily vs. seasonal niches. 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. 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 and the reproductive interference 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 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)). 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 enabling such allochronic speciation (Hendry and Day (2005)) 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 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)). Non-overlapping timing of courtship may then reduce the deleterious effects of male-male competition. In species where female-attracting vocal cues are performed by males, such as in birds or frogs, non-overlapping timing of calls are observed among males (Brumm (2006);

Grafe (1996)). Negative frequency-dependent selection may indeed favor the evolution of divergent calling times in males (Danley et al. (2007)). 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 (1997-06-12)) can be limited to a very short period of the day (Lamarre et al. (2015)). For instance, intense male-male competition has been reported in blue-banded *Morpho* butterflies, including aggressive behaviors both within and between sympatric species (Le Roy et al. (2021)) and the timing of patrolling behavior exhibited by males differs between these sympatric species, therefore suggesting that male-male competition might contribute to the evolution of divergence in temporal niches and eventually to the speciation process itself. Such male-male interactions may again generate negative density dependent selection on the timing of reproductive activities. In a few model investigating the evolution of seasonal patterns of reproduction, as observed in flowering plants, negative density dependence acting on activity time distribution, for instance generated by pollinator limitation, is shown as a potential driver for allochronic speciation (Devaux and Lande (2009); Ye et al. (2014)). Competition in plant species visited by the same pollinator was shown to be strong enough to cause divergence in blooming periods, in accordance with negative frequency dependent selection (Pleasants (1980)). Competition for accessing suitable mating partner can therefore be a driver for divergent evolution of timing of activities linked to reproduction.

The genetic basis underlying variation in the timing of reproductive activities has scarcely been investigated. Nevertheless, in *Drosophila*, the circadian genes *period* and *timeless* were shown to control the daily mating hours and the sister-species *D. simulans* and *D.melanogaster* observed in sympatry exhibit 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 reproductive activities between populations (Wang et al. (2022)). The heritability of this timing is observed in a few species (Evans et al. (2020); Hau et al. (2017)). 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 main driver of speciation is then challenging. Convincing evidence for allochrony causing speciation or reinforcement is nonetheless reported in birds such as *Oceanodroma castro*, where sympatric but genetically isolated populations show asynchronous breeding timings (Friesen et al. (2007)), fish (in *Oncorhynchus gorbuscha* where populations alternatively mate every two years, showing genetic isolation in Limborg et al. (2014)), insects (Joy and Crespi (2007)) and fungi (Kiss et al. (2011)). Allochrony is also shown to be an important factor fueling 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.

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 in a model of neutral speciation triggered by isolation by distance (Baptestini et al. (2013)). How much the interaction between genomic barriers 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 pop-

ulation divergence and speciation. We assumed an haploid species, with two separate sexes, obligatory sexual reproduction and asymmetry in the frequency of mating between males and females. To investigate the interaction between the evolution of timing of activity and genetic barriers to reproductive isolation, we also simulated the evolution of B biallelic 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 identifying the evolutionary mechanisms promoting the emergence of divergent populations with segregated timings of reproductive activities.

Methods

Model overview

We built a stochastic, individual-based birth-and-death process (*e.g.* Sylvie (2016); Otto and Day (2011)), to study the evolution of daily periods of mate searching activity within species, and its potential effect on population divergence and speciation (see table 1 for the parameter list). We assume unbalanced investment in reproduction in males and females: males can mate indefinitely, while females mate a limited number p of times. The virgin females thus become the limiting factor for reproduction, generating intense male-male competition. We considered a population of holometabolous insects, where the imago emerges from the pupae only within a restricted period of the day, as observed in Drosophila flies for instance (Brett (1955)). The emergence timing therefore generated a burst of virgin females at a fixed given period within each day, centered around a parameter e. We then assume that the timing of reproductive activity is a continuous trait, named h_a, with co-dominant inheritance. We used numerical simulations to track down the evolution of distribution of the h_a trait in a population, where mutations may happen on the locus controlling this trait. In our model, each individual of the population thus has a

set of attributes (see table 2), such as its sex and reproductive activity timing h_a, which impact its behavior during the simulation. We simulated death, mating and the emergence events within each day, and followed the distribution of mating activity timing h_a within a population with continuous generations lasting numerous days. We considered each day as a continuous time period, from the sunrise ($t = 0$) to the sunset ($t = 1$). We also tested the case of non-continuous generations, analogous to a *seasonal* model with a non-perennial species.

All individuals are exposed to a baseline death rate d during their adulthood, corresponding to death caused by factors like predation or senescence. We assumed that individuals have a resting/feeding period and a reproductive period each day. 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, which we represent via an activity cost (named δ_e). This cost is applied to both males and females. Because males also engage in competition against each other, we implement another supplementary cost for this male-male competition (named δ_c), increasing their death rate during their activity period. Note that the activity cost (δ_e) affects both males and females and depends on the duration of the activity period within each day. In contrast, the competition cost (δ_c) is specific to males and is determined by the number of males simultaneously active. Egg-laying is also likely to generate a metabolic cost to females: we thus assumed that the more eggs a female lays, the more likely she is to die, represented by an egg-laying cost (named δ_l).

We assume that virgin females probability of mating depends on the encountering rate β with active males (see table 1). 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 mean offspring number per female per day), on the carrying capacity of the environment (K) (see table 1), and on the number of mated females alive at the start of the day ($N_m^{♀}$). The number of offspring per female is drawn individually following a Poisson law of parameter λ_p such that:

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

Note that λ_p is positive in all our simulations, as we do not encounter the fringe cases where $N_m^{♀} > K$.

On the days following the mating event, the time at which each offspring emerges depends on the mean emergence time e with the variance v_e, which are both fixed within each simulation. The emergence time of each individual is then drawn in a truncated Normal law of parameters (e, v_e) bounded in $[0, 1]$.

Individual attributes

Each individual in the population has a set of attributes (listed in table 2). In females, we record the fertilization status, describing whether the female is virgin ($f = 0$) or mated ($f = 1$). A female is considered to be mated once she reaches p reproduction events. We also count the number of eggs laid by each female individual to apply the egg-laying cost δ_l.

The activity period of each individual, *i.e.* the period of the day where the individual is actively trying to mate, is described by the center of its reproductive activity period h_a and the range of its period w_a. w_a is fixed at a value of 0.15 throughout this paper, to avoid having too many varying parameters.

We also assume B biallelic, unlinked, haploid loci that may influence the mating success, within the genome of each individual. In each of these haploid B loci, two alleles (0 or 1) can occur, and the viability of the offspring of each mating depends on the genetic distances across these B loci between the parents. This genetic distance was computed using the Hamming distance (Baptestini et al. (2013)), between the genomes of two individuals x and y in the population 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 divergence between two individuals. In simulations were genetic incompatibilities are implemented, we set a threshold value G above which mating between individuals with $D_H > G$ does not generate any viable offspring. This models a reproductive barrier among genetically divergent individuals.

When a mating event occurs, the attributes of the offspring are set up as follows:

- The sex of the offspring is randomly chosen, assuming a balanced sex-ratio at birth. If the offspring is a female, her fertilization status is initially set to 0.
- In cases where the mother has mated with multiple males ($p_n > 1$), one of them will be chosen uniformly at random as the sire to the offspring.
- We assume that the sexual activity time is a quantitative trait, and that the activity time of both parents will affect the activity time of the offspring evenly. We also consider mutations occurring in the genes controlling the circadian activity, assuming a mutation size μ. We thus set the sexual activity timing parameters of the offspring, h_a (table 2), using a truncated Normal distribution with parameters ($\frac{h_a^{father}+h_a^{mother}}{2}$, μ), bounded in $[0, 1]$.
- For the B loci, we assumed a 50% chance to inherit either parental allele at each locus. Each of the B loci can thus freely recombine and independently mutate with a probability of μ_G.

Events occurring within each day

The day (a continuous interval from sunrise ($t = 0$) to sunset ($t = 1$)) is split in smaller time intervals at the start of day. These time intervals are defined by the temporal position of different events, classified in two categories:

- *Known* events, which can be determined at the start of the day. They are the transitions from resting to reproductive activity (and *vice versa*) for every individual, as well as the emergence of new adult individuals. They are thus all defined before the day start.

- *Unknown* events can happen throughout the day, between two *known* events. They are events that are dependent on the state of the population at a certain moment in time, such as *mating* or *death* events. Those events are drawn using random exponential variables based on the current numbers of active males ($N_a^{♂}$), resting males ($N_r^{♂}$), virgin active females ($N_v^{♀}$), resting virgin females ($N_r^{♀}$), and mated females ($N_m^{♀}$) .

Unknown events occurrence times are computed using the instantaneous mating rate (λ_r) and the instantaneous death rate (λ_d), given by:

$$\lambda_r = \beta \cdot N_a^{♂} \cdot N_v^{♀} \tag{3}$$

and

$$\lambda_d = d(N_a^{♂} + N_r^{♂} + N_v^{♀} + N_r^{♀} + N_m^{♀}) + \delta_e \cdot (N_a^{♂} + N_v^{♀}) + \delta_c \cdot N_a^{♂} \cdot (N_a^{♂} - 1) + \delta_l \cdot \sum_{i=0}^{Nm} E_i. \tag{4}$$

At time t, the probabilities that the next event is a *mating* or a *death* event knowing the occurrence time of the next event are given by $\frac{\lambda_r(t)}{\lambda_r(t)+\lambda_d(t)}$ and $\frac{\lambda_d(t)}{\lambda_r(t)+\lambda_d(t)}$ respectively (proportionally of the exponential variables parameters).

The individual involved in an event is randomly chosen, uniformly among individuals exhibiting the same status. The occurring time of the next event is drawn in an exponential law of parameter λ, with $\lambda = \lambda_r + \lambda_d$. If this occurring time is smaller than the occurring time of the next known event, then a mating or a death event happens, else nothing happens except the next known event. To determine which event between mating and death occurs, a value is drawn from a uniform distribution $U(0,1)$ and is compared to $\frac{\lambda_r}{\lambda}$. If the value drawn is smaller then a mating occurs, we compare it with $\frac{\lambda_r}{\lambda} + \frac{d \cdot N_i^{♂}}{\lambda}$, which corresponds to the death of a inactive

male. If the value is still greater, we compare with $\frac{\lambda_r}{\lambda} + \frac{d \cdot N_i^{♂}}{\lambda} + \frac{N_v^{♀}(d+\delta_e)}{\lambda}$ (death of a virgin active female), then with $\frac{\lambda_r}{\lambda} + \frac{d \cdot N_i^{♂}}{\lambda} + \frac{N_v^{♀}(d+\delta_e)}{\lambda} + \frac{d \cdot N_r^{♀}}{\lambda}$ (death of a virgin resting female). In the case of mated females, we take into account the number of eggs laid by each individual. As such there are N_m events we need to check:

We compare the value drawn from the uniform distribution to $\frac{\lambda_r}{\lambda} + \frac{d \cdot N_i^{♂}}{\lambda} + \frac{N_v^{♀}(d+\delta_e)}{\lambda} + \frac{d \cdot N_r^{♀}}{\lambda} + \frac{d+\delta_l \cdot E_1}{\lambda}$ for the death of mated female number 1, to $\frac{\lambda_r}{\lambda} + \frac{d \cdot N_i^{♂}}{\lambda} + \frac{N_v^{♀}(d+\delta_e)}{\lambda} + \frac{d \cdot N_r^{♀}}{\lambda} + \frac{2d+\delta_l \cdot (E_1+E_2)}{\lambda}$ for the death of mated female number 2 and so on until the event of the death of mated female number N_m: $\frac{\lambda_r}{\lambda} + \frac{d \cdot N_i^{♂}}{\lambda} + \frac{N_v^{♀}(d+\delta_e)}{\lambda} + \frac{d \cdot N_r^{♀}}{\lambda} + \frac{d \cdot N_m^{♀}}{\lambda} + \frac{\delta_l \cdot \sum_{i=1}^{Nm} E_i}{\lambda}$. Finally, if the drawn value is still greater, the last possibility is the death of an active male.

This simulation process is inspired by the Doob-Gillespie algorithm (see Gillespie (1977); Tran (2006)). Note that the more active individuals there are at the same time, the lower is the individual chance of reproduction at that time.

To compare the results of our model investigating daily activity to one investigating seasonal activity, we slightly modified our simulations, by assuming non-overlapping generations, *i.e.* assuming that all the adult individuals die after each time unit. In the previous model (referred to as *daily* model hereafter), the time unit considered for a generation was a day, and in this seasonal version (referred to as *seasonal* model hereafter), each time unit is considered to be a year. Thus in the following time unit, offspring generated from reproduction within the previous generation will again reach sexual maturity (at a time $t = e$) and mate afterwards if possible.

Simulation outcomes

To detect the evolution of differentiated populations during the course of a simulation, we track down the distribution of the trait h_a throughout all individuals. We aim at detecting the number of peaks in this distribution, using the *find_peaks* function from the "scipy.signal" package. We set the *find_peaks* parameters to 0.5 for the prominence and 30 for the distance. This ensures that the

sub-populations have separated enough temporal niches and that our peaks are not due to low-scale stochastic variations. A group of individuals with similar timing of activities that diverge from the ancestral population is then considered as differentiated population only when (1) it lasted more than forty generations within the simulation and when (2) the position of the peak within the differentiated population did not vary by more than 0.15 units of time over a generation. In our simulations, we only observed cases where we had either one or two peaks, meaning we either had one or two differentiated sub-populations, no matter the set of parameters used. When two peaks are detected, we could categorize them in two categories: *early* sub-populations when the average reproductive activity timing of the sub-population was below $t = 0.3$, and *late* sub-populations otherwise. We only had at most one of each sub-population type at a time, and did not observe two *early* or *late* sub-populations at the same time in the *daily* model.

When differentiated sub-populations are observed at the end of a simulation, we estimated the level of genetic differentiation between the two populations, using the F_{st} values computed from genetic variations observed at the B loci. The F_{st} is calculated according to the following equation, from Hudson et al. (1992):

$$F_{st} = \frac{\pi_{inter} - \pi_{intra}}{\pi_{inter}} \tag{5}$$

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.

We first tested the effect of multiple parameters on the emergence of differentiated sub-populations.

We simulated cases with varying values of the emergence time of adult individuals e, and differing values of the associated variance v_e. All other parameters remained fixed in those simulations, with values set-up to allow the persistence of stable populations within the simulations. We then compared the respective effects of the cost of male-male competition with the cost of male-female asymmetry in mating events on the evolution of differentiated populations. We ran simulations with different values of the strength of male-male competition (δ_c), and of the number of possible mating events throughout the life of females (p).

Next, we implemented genetic incompatibilities in our model, to evaluate the strength of the genetic differentiation between subpopulations using a F_{st} metric. To assess this differentiation, we ran simulations with varying values of G and δ_c.

We also studied the stability of the coexistence between *early* and *late* sub-populations, with varying values of K, G and δ_c. Finally, we compared simulations assuming either a *daily* or a *seasonal* model of temporal niche evolution, for different values of the adult emergence time (e) and strength of male-male competition (δ_c).

A hundred replicates were run for each condition and the range of parameters used is given in table 1.

Python (3.11.7) was used for modeling purposes and data cleaning, using the packages "random", "numpy", "scipy". R (4.4.0) was used to plot all results, using the "ggplot2" package.

Numerical simulations and equilibria

We ran each simulation for $N = 500$ generations for each repetition, ensuring that the equilibrium is reached for simulations (confirmed this with simulations such as fig. S1). We used the parameters and attribute range values provided in tables 1 and 2. We also tracked down the evolution of the activity period trait h_a, and the genotypes at the B loci across all individuals.

The initial conditions for all simulations are a population of hundred individuals with a balanced

sex-ratio. All females are initially virgin. The initial values of h_a and of all males are set with truncated in [0,1] Normal laws of parameters (0.5, μ) and (0.05, μ) respectively. The genomes are initialized with all values equal to 0.

Results

Evolution of sub-populations with earlier reproductive activity

Assuming that females are able to mate maximum once throughout their life, and assuming no genetic incompatibilities, our simulations result in either a unimodal or a bimodal distribution of the timing of reproductive activities, depending on the timing of emergence of adults e (fig. 1).

- When a unimodal distribution of activity was observed, the average timing of reproductive activity tightly follows the timing of emergence for most e values (fig. S2), until a certain threshold ($e \geq 0.8$) where individuals are not active at dusk, but rather at dawn. 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 an *early* niche and one with a *late* niche) can co-exist. The sub-population with the *early* niche has an average timing of reproductive activity close to the start of the day, while the sub-population with a *late* niche displays an average timing of activity close to the moment of adult emergence, where more virgin females are available. (fig. S2).

When the available temporal niche was reduced (*e.g.* when the emergence of adults occurs very early or very late), the proportion of simulations where a bimodal distribution was observed

decreased (fig. 1). The evolution of differentiated populations with different timings of reproductive activities is thus more likely to happen when adult emergence occurs in the middle of the day, as it provides more opportunity for temporal niche differentiation earlier during the day.

Variation in the timing of adult emergence shapes population differentiation

Because the variability in the timing of adult emergence (v_e) throughout the day can impact the probability of encountering between males and virgin females, we then run simulations for different levels of variability in the timing of emergence, assuming that females can only mate once, and followed the distribution of the average activity timing trait $\overline{h}_a$ through time.

Assuming that adults emerge around noon ($e = 0.5$), we run 100 simulations for each level of variation in the timing of the emergence and observed three main evolutionary outcomes (fig. 2):

- When assuming high variability in the emergence timing ($v_e > 0.12$), a unimodal distribution of timing of reproductive activity is observed, with sexual activities occurring early in the morning, away from the mean emergence time ($e = 0.5$). The high uncertainty in the timing of availability in virgin females likely promotes the evolution of the earliest possible timing of reproductive activities.

- When assuming low variability in the emergence timing ($v_e < 0.04$), a unimodal distribution of timing of reproductive activity is also observed in a majority of simulations, but with sexual activities occurring later in the day, around the mean emergence time ($e = 0.5$). The low variance in the timing of availability in virgin females likely hinder the evolution of individuals with earlier activity timings.

- When assuming moderate levels of variations in the emergence timing ($0.04 \leq v_e \leq 0.12$), most simulations result in a bimodal distribution of the sexual activity timing, generating sub-populations with different timings of reproduction. In that case, one sub-population had a timing of sexual activity early during the day while temporal niche of the alternative

population was centered close to the time of emergence, as previously observed (see fig. S2).

Variation in the timing of adult emergence within population thus significantly affects the evolution of temporal niches.

Male-male competition and male-female asymmetry promoting the evolution of differentiated sub-populations

Since male-male competition could generate negative frequency dependent selection on the timing of male sexual activity, we test whether the strength of this competition (δ_c) impacts the evolution of sub-populations with different timings of sexual activities. We compare the proportion of simulations where a bimodal distribution of the timing of activities was observed at N=500 generations, when assuming different levels for the cost of male-male competition. Fig. 3A shows an increase of the probability of differentiation into two sub-populations when low competition exists ($\delta_c \leq 0.05$), compared to simulation without competition. However, when the competition becomes too intense ($\delta_c > 0.05$), the probability of observing differentiated populations decreases, reaching lower values than simulations without competition (nearing zero when $\delta_c = 1$).

Our model thus suggests that the cost of competition between males can favor the evolution of differentiated timings of reproductive activities, because males with unusual timings of activity might benefit from reduced competition.

Nevertheless, the evolution of differentiated sub-populations is still observed when male-male competition has no cost ($\delta_c = 0$, fig. 3A). In our initial simulations, we indeed assumed that females could mate only once ($p = 1$), while males can mate several times throughout their lives, and this asymmetry alone can generate negative frequency-dependent selection on the timing of reproductive activities.

When comparing simulations allowing either a maximum of one mating event per female ($p = 1$) *vs.* multiple mating events per female (from $p = 2$ to $p = 5$) (fig. 3B), we 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 decreases, from *circa* 75% when $p = 1$, to being almost never observed when $p = 5$. Female mating limitations thus strongly favors the emergence of bimodal distributions in the reproductive activity timing of individuals. Our model therefore suggests that intense male-male competition for access to females is likely to promote the evolution of differentiated reproductive temporal niches in sympatry.

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 G, above which reproduction between pairs of individuals is unsuccessful. fig. S3 shows that G has a strong effect on the probability of evolution of differentiation and coexistence of sub-populations with divergent temporal niches:

- When the threshold G is low, *i.e.* when one to three allelic variations between parents at a single locus are sufficient to prevent the generation of viable offspring, the evolution of differentiated sub-populations is drastically limited. Such drastic incompatibilities may strongly limit reproduction and thus prevents the evolution of unusual timings of reproductive activity. In extreme cases ($G = 0.1$), every simulation ends in an extinction, because most individuals failed to generate offspring. For $G = 0.2$, only 45% of simulations ended with an extinction, while no extinction is observed for higher levels of G.

- When assuming intermediate values ($0.4 <= G <= 0.6$), 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 totally lacking.

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, promoting the differentiation between *early* and *late* sub-population. For $G = 0.2$, we observe a high variability in F_{st} values among simulations. This may be due to smaller population sizes (due to reproduction being drastically limited) enhancing genetic drift and thus inflating F_{st} values. 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. S3.

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 doubled in simulations with high competition ($\delta_c = 1$) as compared to simulations without ($\delta_c = 0$).

Interestingly, for high values of δ_c where the probability of observing differentiated populations decreases (see fig. 3A), F_{st} values continue to increase: in the few cases where sub-populations do evolve, high levels of genetic differentiation are favored by the intense level of competition, counter-selecting intermediate values of h_a.

Genetic differentiation between sub-populations thus appears to be positively correlated to both the strength of male-male competition and of incompatibilities between divergent genomes. Nevertheless, we did not detect any link between the percentage of simulations ending with sub-populations with different timings of reproductive activity and the average genetic differentiation

between those sub-populations for the same parameter combinations.

Ecological factors shaping the coexistence between sub-populations

In most simulations, *early* subpopulations frequently evolved and got extinct during the course of a simulation. We thus investigated the factors that may impact the extinction of the *early* subpopulations during the simulations. We tested the effect of the carrying capacity of the environment (K), male-male competition (δ_c) and genetic incompatibilities (G) on coexistence of *late* and *early* sub-populations.

For simulation length purposes, we first used a low carrying capacity of the environment at $K = 1000$ individuals in most simulations. To test the impact of this parameter, we then ran a series of simulations assuming higher carrying capacities ($K = 2000$ and $K = 5000$), but did not detect significant difference between simulations (fig. 5A). For the genetic threshold of reproductive isolation G, we found that a value of $G = 0.5$ resulted in longer coexistence duration. The duration of coexistence decreases when either $G > 0.5$ or $G < 0.5$. Notably, when $G = 1$, akin to simulations without any incompatibility, coexistence had much shorter duration. Genetic differentiation thus favors the coexistence of sub-populations through time, by limiting gene flow between them. Finally, for the strength of male-male competition, we found results similar to the probability of differentiation in two sub-populations in fig. 3A. When low competition was assumed ($\delta_c < 0.05$), the duration of coexistence was on average higher compared to simulations without competition ($\delta_c = 0$), but decreases as the strength of male-male competition increases. When competition gets too intense ($\delta_c \geq 0.5$), the duration of coexistence became lower than simulations assuming no competition, because of intense competitive exclusion between the two sub-populations.

Seasonal versus daily allochrony

To compare the evolution of divergence in the timing of reproductive activity at the *daily vs. seasonal* scale, we then compared simulations assuming over-lapping *vs.* non-overlapping generations respectively. In the simulations assuming non-overlapping generations (*i.e. seasonal* model), we observe that, similarly to the *daily* model, the evolution of the reproductive activity time was strongly driven by the emergence timing e (fig. S4). However, in the *seasonal* model, whatever the e value, and assuming the level of competition between males previously used in simulations assuming the *daily* model ($\delta_c = 0.1$), bimodality in the distribution of the reproductive activity timing was never observed at the end of simulations (see fig. S5). In the *seasonal* model, individuals cannot be reproductively active before their timing of adult emergence, unlike in the *daily* model where they can search for mates the next morning, earlier activity patterns are probably strongly counter-selected in the *seasonal* model, and reproductive timing evolved toward values close to the emergence time e.

We then run simulations with larger strength of male-male competition and genetic incompatibilities in the *seasonal* model. Similarly to the *daily* model, the negative frequency dependent selection, fueled by male-male competition, can promote the evolution of differentiated temporal niches, but only for extreme values of δ_c (fig. S6A). We observe up to *circa* 60% of simulations with coexisting sub-population in our *seasonal* model, notably lower than in simulations assuming the *daily* model, where we reach almost 100% of simulations with differentiated sub-populations (fig. 6). Interestingly, when differentiated populations were observed in the *seasonal* model, the timing of reproductive activities of the two sub-populations differed from the *daily* model. We indeed observed the evolution of a *later* sub-populations (fig. 6B and S6B), coexisting with a sub-population with the temporal niche located immediately after the time of adult emergence. As the cost of competition increased, the activity timings of the *later* sub-population became closer to the end of the season. This strongly departs from the previous results obtained assuming *daily*

model (as seen in fig. 6A), where new sub-populations have a temporal niche *earlier* than the sub-population active around the time of adult emergence. Since being active earlier than emergence is not a possibility in the *seasonal* model, individuals with later activity timings become favored, as they suffer from reduced survival costs incurred by male-male competition.

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. The differentiation into different temporal niches is mainly due to the limited availability in virgin females, but is also promoted by costs generated by male-male interactions. Negative density-dependent selection acting on the timing of reproductive activities can therefore promote differentiation into different temporal niches. 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 virgin females. However, in the *seasonal* model, for similar sets of parameters, extreme values of male-male competition promoted the evolution of differentiated sub-populations with later activity timings. Our *seasonal* model therefore does not fit with to the protandry actually observed at the seasonal scale for many species, from flowering plants (Zhao et al. (2020)) to butterflies (Forsberg and Wiklund (1988)). Here we indeed did not focus on the evolution of sexual maturity, but on the evolution of the timing of reproductive activity independently from the development until sexual maturity. In models studying the evolution of protandry (*e.g.* applied to flowering time in plants (Forrest (2014)) or to emergence time in

butterflies (Iwasa et al. (1983))), the evolving trait is the timing of sexual maturity, akin to the parameter e in our model and the timing of reproductive activity is entirely controlled by the development time until sexual maturity. In our model however, the timing of reproductive activity (h_a) can evolve independently from the timing of sexual maturity acquisition (e). We therefore observed here strong discrepancy between the *daily* model, where sub-populations with reproductive activities occurring earlier can evolve with the *seasonal* model where only sub-population with delayed reproductive activities can be observed. Two mains factors are at play in the emergence of *late* temporal niches in our *seasonal* model: (1) individuals could not be active before the emergence timing, due to the non-overlapping generations assumed, and (2) male-male competition needed to incur a high cost so that individuals active later in the season had a better fitness, despite reduced access to virgin females. Daily and seasonal temporal niches for reproductive activity thus may be under different evolutionary constraints, especially in the case of non-perennial seasonal species.

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 heritable, which is supported by evidence of studies on the daily free-running periods in birds (with *Parus major* in Helm and Visser (2010)), or crickets (with *Dianemobius nigrofasciutus* in Shimizu and Masaki (1997)). These studies show high heritability of this key property of the circadian clock, with h^2 values for this trait as high as 0.86 ± 0.24. Genetic evidence for allochrony on a daily basis can also be found in mice species (Wang et al. (2022)), with divergence in genes related to circadian rhythm control. 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 behaviour, 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 carrying capacity K, 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. Temporal divergence may thus be promoted by both male-male competition and other ecological factors.

Abiotic factors such as temperature also may also enhance allochronic speciation, if the re-

productive 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 both throughout a day and a year, 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 seem mandatory to generate sub-populations differentiated on a genetic level, as the gene

flow between sub-population keeps occurring in absence of these genetic incompatibilities. By testing the proportion of loci G required for incompatibilities, we found that intermediate values seemed to promote the longevity of *early* sub-populations. These values seem to allow a small 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 reinforcement 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 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 ancestral population and subject to too much male-male competition. 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)). 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 and Code Availability

All code and data is available anonymously at https://anonymous.4open.science/r/Daily-niche-model-0D05.

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Tables

Table 1: Parameters of the model and baseline values used in numerical simulations

Parameter	Description	Values
β	Encountering rate between active males and virgin 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	$0, 0.01, 0.02, ..., 0.2$
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	$0.01, 0.02, ..., 0.2$
μ	Mutation size of the reproductive activity period timing	0.04
μ_G	Mutation rate of each neutral gene	0.1
G	Genetic threshold of reproductive isolation	$0.1, 0.2, ..., 0.8, 0.9$
K	Carrying capacity of the environment	$1000, 2000, 5000$

Table 2: 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
f	Female fertilization status	0 or 1
p_n	Current number of mating attempts by a female	$0 \leq p_n \leq p$
E	Number of eggs laid by a mated female	Variable integer
B	Number of loci in the neutral genome	10

Figure legends

Figure 1: **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 generations, for 100 replicates per value of the timing of adult emergence e. (B), (C) and (D) Distributions of the timing of reproductive activities in the different possible outcomes. Two types of unimodal distribution of the timing of reproductive activity can be observed after 500 generations: (B) a single *early* population (represented by stripes), where the average time of reproductive activity is below the value $t = 0.3$ or (C) a single *late* population (represented by dots). Bimodal distribution of timing of activities leading to the co-existence of two sub-populations can also be observed (D), with both *early* and *late* sub-populations, represented in white. The vertical dotted line in (B), (C) and (D) show the$t = 0.3$ threshold, defining *early* and *late* sub-populations. All simulations were run assuming the same parameter values: $\beta = 3, \delta_c = 0.1, G = 1, e = 0.5, V_e = 0.05, p = 1, K = 1000$.

Figure 2: **Effect of variance in the timing of adult emergence on the evolution of sub-populations with differentiated temporal niches**. The percentage of simulations where either one or two sub-populations were observed after 500 generations is shown, for 100 replicates per value of the variance in the timing of adult emergence V_e, assuming that this timing of emergence is centered around noon ($e = 0.5$). When only a single population was maintained, we distinguish *early* (in stripes) and *late* (in dots) populations. Simulations may also result in the coexistence of two sub-populations, with *early* and *late* (in white) timing of reproductive activities. All simulations were run assuming the same parameter values: $\beta = 3, \delta_c = 0.1, G = 1, e = 0.5, p = 1, K = 1000$

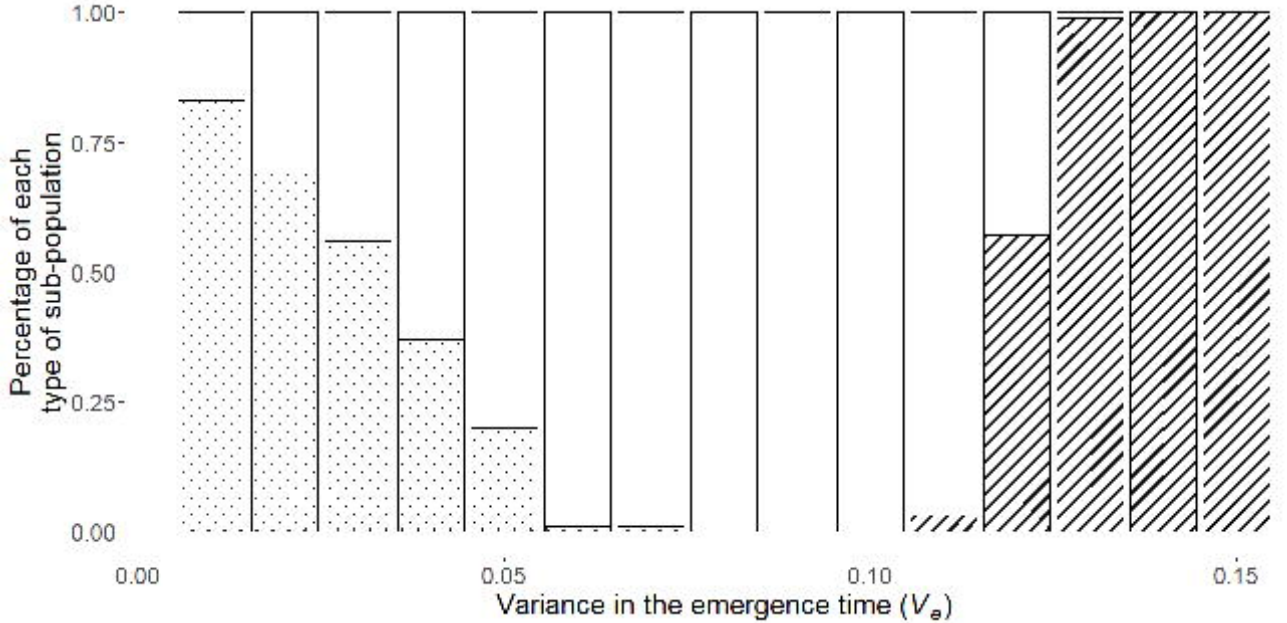
Figure 3: **Effects of the male-male competition and male-female asymmetry on the evolution of sub-populations with different temporal niches.** (A) Effect of the intensity of male-male competition (δ_e) on the percentage of simulations where a bimodal distribution was observed after 500 generations, for 100 replicates per condition. Intermediate values (around $\delta_c = 0.05$) lead to the higher percentage of simulations with coexistence of *early* and *late* sub-populations. Note that x-values are provided using a log_{10} scale. (B) Effect of maximum mates per female on the percentage of simulations where a bimodal distribution was observed after 500 generations for 100 replicates per condition.

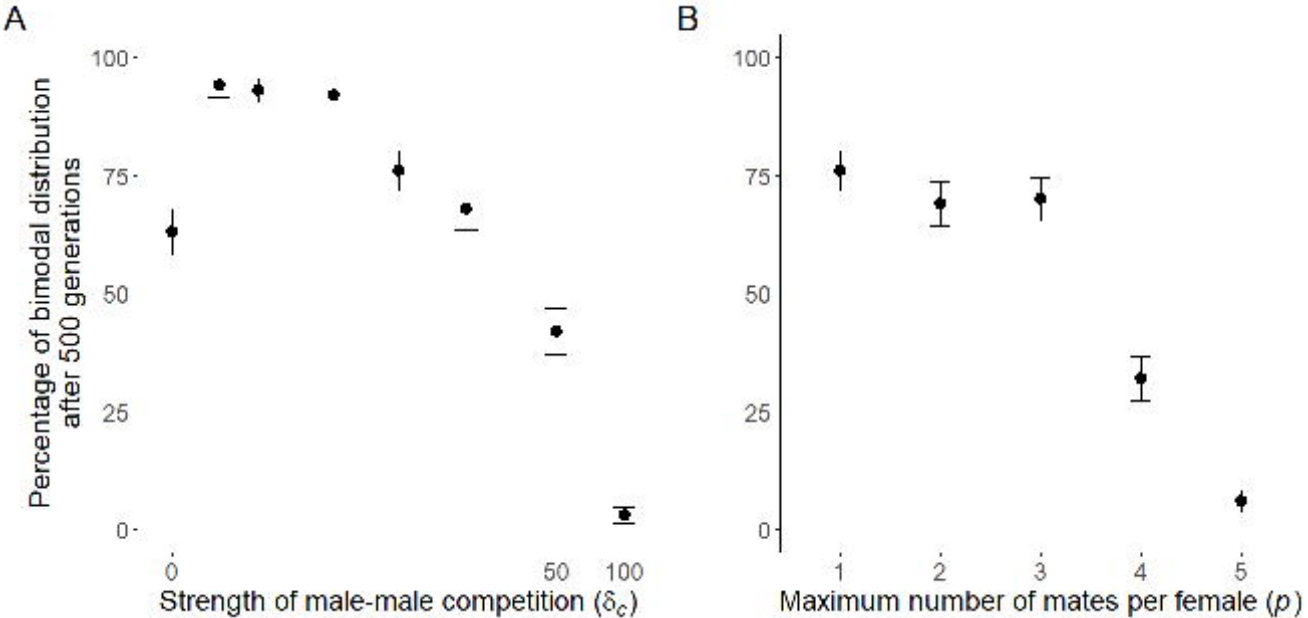
Error bars are one standard deviation (SD). All simulations were run assuming the same parameter values: $\delta_c = 0.1$, except in 3A, $\beta = 3$, G = 1, $e = 0.5, V_e = 0.05, p = 1$, except in 3B, $K = 1000$

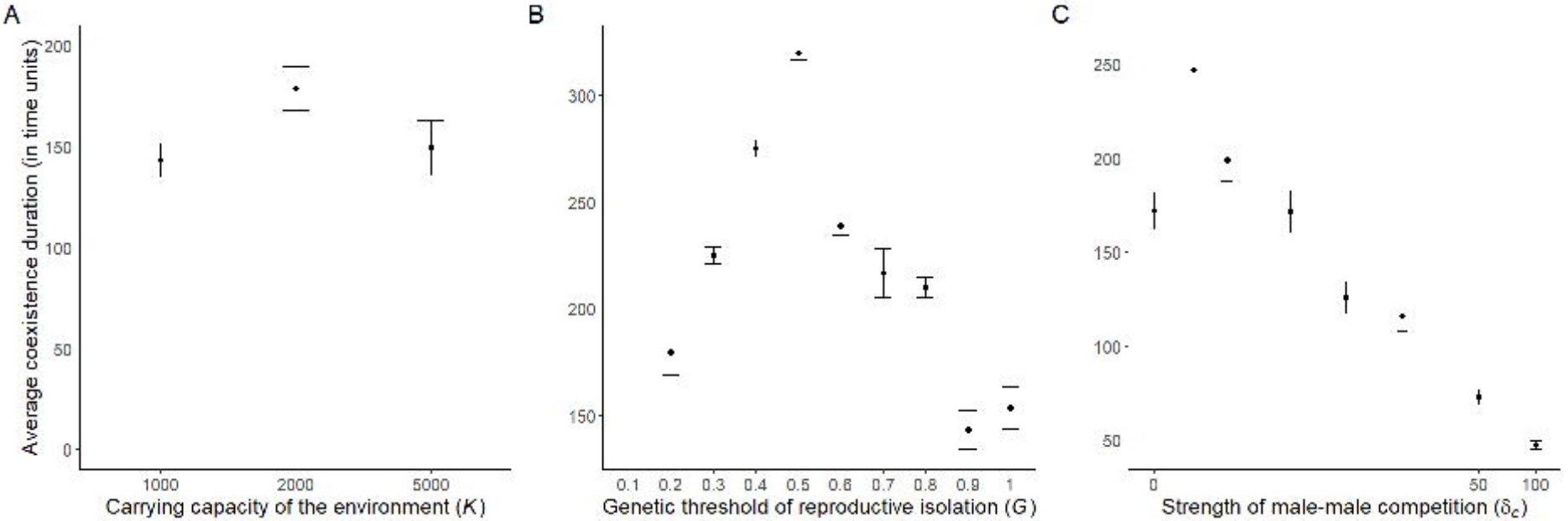
Figure 4: **Effect of genetic incompatibility and male-male competition on the genetic differentiation between sub-populations with different temporal niches.** The average F_{st} between sub-populations where a bimodal distribution was observed after 500 generations, assuming genetic incompatibilities is shown, for 1000 replicates per values. (A) Effect of the proportion of loci G required for mating incompatibilities on the level differentiation between sub-populations, estimated by F_{ST}. While very small values of G do not sustain population differentiation, 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 are a 95% confidence interval. All simulations were run assuming the same parameter values: $\beta = 3, \delta_c = 0.1, e = 0.5, V_e = 0.05, p = 1, K = 1000$

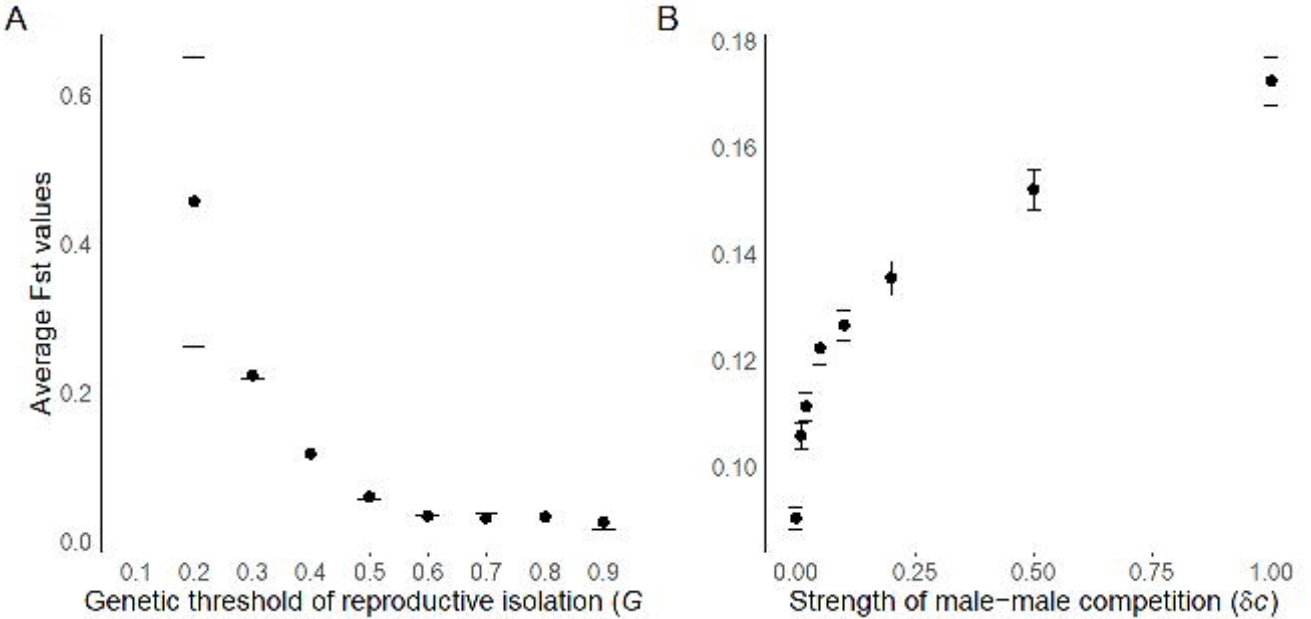
Figure 5: **Factors shaping the duration of coexistence between *early* and *late* sub-populations.** We ran 100 simulations for 500 generations and followed the duration of coexistence between *early* and *late* sub-populations, depending on different parameters: (A) The carrying capacity K. (B) The genetic threshold G of reproductive isolation. (C) The strength of male-male competition. Error bars are a 95% confidence interval. All simulations were run assuming the same parameter values: $\delta_c = 0.1$, except in Fig.5C, $\beta = 3$, $G = 1$, except in Fig.5B, $e = 0.5, V_e = 0.05, p = 1, K = 1000$, except in Fig.5A.

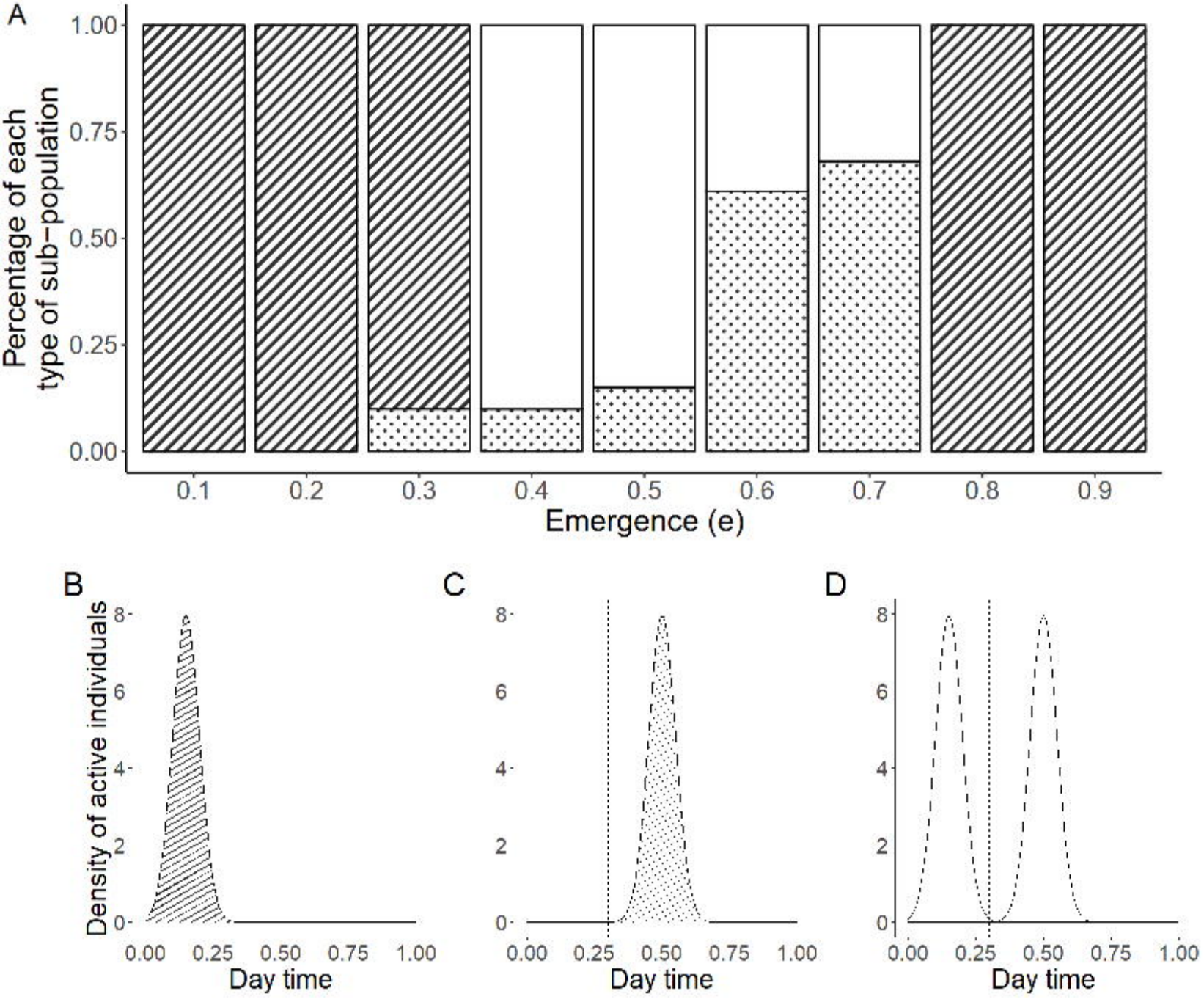
Figure 6: **Comparison of bimodal simulation outcomes, assuming either the *daily* or the *seasonal* model.** Distributions of the timing of reproductive activities when differentiated sub-population evolve in (A) the *daily* model assuming over-lapping generations and $\delta_c = 0.1$ and (B) the *seasonal* model, assuming non -over-lapping generations and $\delta_c = 3$. The emergence time $e = 0.5$ is shown by a dotted line. In simulations assuming the *daily* model, we observe the evolution of a sub-population displaying earlier reproductive activity as compared to the alternative sub-population where reproductive activities occurred around the emergence time. In simulations assuming the *seasonal* model, both sub-populations were active after the emergence time e. All other parameters are assumed to be: $\beta = 3, G = 0.4, V_e = 0.05, p = 1, K = 1000$

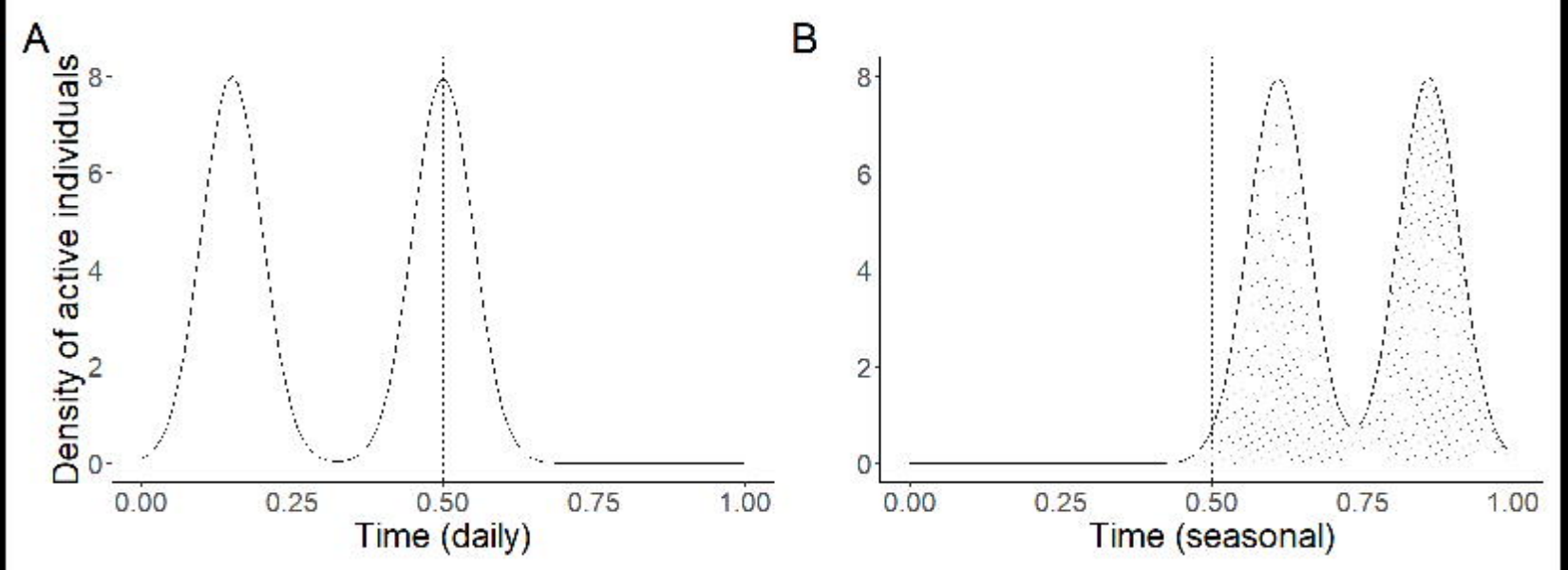

1.00
0.75
0.50
0.25
0.00
Percentage of each type of sub-population
0.00
0.05
0.10
0.15
Variance in the emergence time (V_e)


A
B
Percentage of bimodal distribution after 500 generations
100
75
50
25
0
0
50
100
Strength of male-male competition (δ_c)
1
2
3
4
5
Maximum number of mates per female (p)


A
B
C
Average coexistence duration (in time units)
0
50
100
150
200
1000
2000
5000
Carrying capacity of the environment (K)
150
200
250
300
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Genetic threshold of reproductive isolation (G)
50
100
150
200
250
0
50
100
Strength of male-male competition (δc)


A
Average Fst values
0.0
0.2
0.4
0.6
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Genetic threshold of reproductive isolation (G
B
0.10
0.12
0.14
0.16
0.18
0.00
0.25
0.50
0.75
1.00
Strength of male−male competition (δc)


A
Percentage of each type of sub−population
1.00
0.75
0.50
0.25
0.00
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Emergence (e)
B
C
D
Density of active individuals
8
6
4
2
0
0.00
0.25
0.50
0.75
1.00
Day time


A
Density of active individuals
8
6
4
2
0
0.00
0.25
0.50
0.75
1.00
Time (daily)
B
8
6
4
2
0
0.00
0.25
0.50
0.75
1.00
Time (seasonal)