

1 Insects and incest: sib-mating tolerance in natural populations of a parasitoid wasp

Marie Collet^{1*}, Isabelle Amat, Sandrine Sauzet¹, Alexandra Auguste², Xavier Fauvergue², Laurence Mouton¹, Emmanuel Desouhant¹

¹ Univ Lyon, Université Lyon 1, CNRS, Laboratoire de Biométrie et Biologie Evolutive UMR5558, F-69622 Villeurbanne, France

2 ² INRA, CNRS, Université Côte d'Azur, ISA, France

3 *Corresponding author: marie.collet@univ-lyon1.fr

4

Abstract

5 1. Sib-mating avoidance is a pervasive behaviour that likely evolves in species subject to
 6 inbreeding depression. Laboratory studies have provided elegant demonstrations of sib-
 7 mating avoidance, but small-scale bioassays often minimize the costs associated with mate
 8 finding and choice, which could lead to spurious findings.

9 2. We used the hymenopteran parasitoid wasp *Venturia canescens* as a model organism,
 10 because previous laboratory studies revealed that sib-mating led to a 25% decrease in
 11 fertile offspring, and that sib-mating was partially avoided.

12 3. Our study consisted of a mate choice experiment in laboratory cages to determine if kin
 13 discrimination occurs in this species. We further performed a field study in which 86 wild-
 14 caught males, 155 wild-caught females and their 226 daughters were genotyped at
 15 eighteen microsatellite loci. With these data, we reconstructed the genotype of each
 16 female's mate and estimated the relatedness of each mating pair.

17 4. Mate choice experiments confirmed that females are capable of discriminatjng kin. Time
 18 to mating depended on the frequency of female encounters with related and unrelated
 19 males. Contrary to previously published results, however, no sib-mating avoidance was

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43 detected. In the field, the effective rate of sib-mating did not differ from the probability
 44 that sibs encounter one other at random, which corroborates the absence of sib-mating
 45 avoidance. We also detected a weak but significant male bias in dispersal, which could
 46 reduce encounters between sibs.

47 **5.** Our results suggest that, despite kin discrimination, V. canescens tolerates sib-mating in
 48 the field. This raises the question as to why kin discrimination is maintained in this species.
 49 It further calls into question the idea that inbreeding depression occurs in most species
 50 with single-locus complementary sex determination.

51 **Key-words:** Microsatellites; Inbreeding tolerance, Kin recognition, Parasitic wasp, sl-CSD.

52 Your keywords should only contain words that are not mentioned in the title/abstract. This allows
 53 others to find your work using the big article databases (that search the title, abstract and keywords).

54 Introduction

55 When inbreeding lowers fitness-related components, such as survival and fertility, natural
 56 selection should favour behaviours preventing the reproduction of genetically-related individuals, or
 57 mitigating harmful consequences referred to as inbreeding depression (Pusey and Wolf, 1996; Keller
 58 and Waller, 2002; Angeloni et al., 2011). Inbreeding depression is caused by the expression of
 59 deleterious recessive alleles at the homozygous state or by a loss of heterosis in inbred crosses
 60 (Charlesworth and Charlesworth, 1987; Pusey and Wolf, 1996). Selection on behaviours underlying
 61 inbreeding avoidance should thus depend on inbreeding load, which balances the advantages of
 62 inbreeding avoidance with the costs of implementing adapted physiological or behavioural responses.
 63 Selection may also depend on the benefits of inbreeding in terms of inclusive fitness, as inbreeding
 64 results in an increased representation of genes identical by descent in the offspring (Kokko and Ots,
 65 2006; Puurtinen, 2011; Duthie and Reid, 2016).

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83 Responses to inbreeding risk reflect the benefits and costs associated with inbreeding and
 84 inbreeding avoidance. Observations range from systematic inbreeding avoidance to inbreeding
 85 tolerance or even inbreeding preference (Szulkin et al., 2013). For example, inbreeding preference was
 86 observed in the cichlid fish *Pelvicachromis taeniatus*, where inbreeding does not lower survival and
 87 growth rate of young (Thünken et al., 2007). Related pairs further invest more in parental care and
 88 cooperate better. In rodents, such as *Marmota flaviventris*, inbreeding is preferred despite lower
 89 survival of inbred progeny. This preference can be explained by the large variation of male
 90 reproductive success and the composition of social groups, where males mating with related females
 91 do not suffer decreased annual reproductive success compared to the majority of males with little or
 92 no reproductive success (Olson et al., 2012). In contrast, mole rats or ring-tailed lemurs living in socially
 93 structured groups avoid inbreeding. In mole rats, avoidance leads to a high reproductive skew in males,
 94 with a subset of males that never reproduce (Cooney and Bennett, 2000). In ring-tailed lemurs,
 95 inbreeding avoidance allows females to save energy (i.e. invested in pregnancy and lactation) that
 96 would have been allocated to the production of inbred offspring, which suffer from depressed immune
 97 functions and have a reduced life expectancy (Boulet et al., 2009; Charpentier et al., 2008). It is,
 98 therefore, essential to quantify the costs and benefits of inbreeding avoidance to understand the
 99 consequences of inbreeding and the evolution of avoidance behaviours.

100 Several behavioural and physiological strategies to avoid inbreeding risks have evolved in
 101 animals, including sib-mating avoidance, dispersal (Greenwood et al., 1978; Szulkin and Sheldon,
 102 2008), polyandry, extra-pair paternity, divorce (Hatchwell et al., 2000; Cornell and Tregenza, 2007;
 103 Cohan et al., 2008; Lardy et al., 2011; Reid et al., 2015; Duthie and Reid, 2016), and postcopulatory
 104 mechanisms, such as the preferential use of sperm from unrelated males by inseminated females
 105 (Tregenza and Wedell, 2002; Bretman et al., 2004). Mate choice is probably the most pervasive
 106 behaviour for sib-mating avoidance (Pusey and Wolf, 1996) and relies mostly on sib recognition
 107 mediated by chemical communication (Howard and Blomquist, 2005; Johansson and Jones, 2007;
 108 Charpentier et al., 2008a; Bonadonna and Sanz-Aguilar, 2012). It is nonetheless important to note that

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139 [kin recognition mechanisms can arise from selective forces other than inbreeding avoidance, such as](#)
 140 [nest-mate recognition, parental care, territory defence, and dominance hierarchies among others](#)
 141 [\(Mateo, 2004\)](#). The balance between [the costs and benefits](#) associated with inbreeding avoidance also
 142 depend on environmental factors, such as population density, spatial or social population structure. A
 143 low population density constrains mate availability and [encounter rates](#) and may, therefore, influence
 144 mate choice (Kokko and Rankin, 2006; Duthie and Reid, 2016). In the lizard *Zootoca vivipara*, [for](#)
 145 [example](#), female choosiness is reduced when mate encounter rates decrease (Breedveld and Fitze,
 146 2015). [Moreover](#), in the marsupial carnivore, *Antechinus agilis*, habitat selectivity following natal
 147 dispersal is negatively correlated with the abundance and relatedness of females occupying [the novel](#)
 148 [habitat](#) suggesting a pervasive effect of inbreeding risk on dispersal (Banks and Lindenmayer, 2014). In
 149 cooperatively breeding species, inbreeding avoidance strategies can lead to the suppression of
 150 reproduction outside dominant pairs due to the lack of opportunity [For](#) outbred matings (as in the
 151 Damaraland mole-rats *Fukomys damarensis*; Cooney and Bennett, 2000). [Reproductively](#) suppressed
 152 subordinates that are related to the dominant pair should, [therefore](#), increase their involvement in
 153 [raising offspring](#) to increase their inclusive fitness and maintain the social structure of the group
 154 (Nichols, 2017). [The importance of environmental factors in shaping inbreeding avoidance thus](#)
 155 [requires field data to be able to quantify costs and benefits associated with each strategy.](#)

156 [Assessing](#) inbreeding avoidance patterns, that is, the occurrence of inbreeding avoidance and
 157 behavioural strategies implied, is a difficult task that requires the estimation of relatedness coefficients
 158 between actual and potential mates (Szulkin et al., 2013; [This reference is not in the bibliography](#)). This
 159 may explain why most field studies have been conducted on large species of mammals and birds, for
 160 which monitoring is much easier compared to small invertebrates (Cohas et al., 2008; [Herfindal et al.](#),
 161 2014; [Arct et al.](#), 2015; [Hardouin et al.](#), 2015). It is [thus](#) not surprising that inbreeding avoidance
 162 patterns have been rarely documented in insects in the wild [\(but see Robinson et al., 2012 and Bretman](#)
 163 [et al., 2009](#) [This former reference is not in your bibliography. I've included the reference of Bretman](#)
 164 [2009, because they did a long-term genetic study over several years and throughout the season in a](#)

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Supprimé: Various behavioural and physiological strategies to avoid inbreeding risks have been selected for in animals, including sib-mating avoidance, dispersal (Greenwood et al., 1978; Szulkin and Sheldon, 2008), polyandry, extra-pair paternity, divorce (Hatchwell et al., 2000; Cornell and Tregenza, 2007; Cohas et al., 2008; Lardy et al., 2011; Reid et al., 2015; Duthie and Reid, 2016), and postcopulatory mechanisms, such as the preferential use of sperm from unrelated males by inseminated females (Tregenza and Wedell, 2002; Bretman et al., 2004). Mate choice is probably the most pervasive behaviour for sib-mating avoidance (Pusey and Wolf, 1996) and relies mostly on sib recognition mediated by chemical communication (Howard and Blomquist, 2005; Johansson and Jones, 2007; Charpentier et al., 2008a; Bonadonna and Sanz-Aguilar, 2012). It is nonetheless important to note that kin recognition mechanisms can arise from selective forces other than inbreeding avoidance, such as nest-mate recognition, parental care, territory defence, dominance hierarchies to name a few (Mateo, 2004).
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214 [wild insect population](#)). Laboratory studies nonetheless suggest various strategies ~~for~~ inbreeding
 215 avoidance in different insect ~~genera~~, such as precopulatory avoidance of related males ~~in butterflies~~
 216 (~~Fischer et al., 2015~~) ~~and~~ postcopulatory choice, ~~where stored sperm from unrelated males is used~~
 217 ~~preferentially by females of the cricket *Gryllus bimaculatus* (Bretman et al., 2004, 2009)~~. In a social
 218 species of termite, *Neotermes chilensis*, dispersal of ~~colony~~ founding ~~individuals~~ is the main mechanism
 219 to avoid inbreeding (Aguilera-Olivares et al., 2015). ~~In contrast~~, ~~absence~~ of inbreeding avoidance has
 220 also been ~~documented~~ in insects, ~~including~~ parasitoid wasps (Bourdais and Hance, 2009) ~~and~~
 221 *Drosophila melanogaster* (Tan et al., 2012). The diversity of strategies unveiled in ~~laboratory~~
 222 ~~experiments calls for a more thorough investigation of~~ inbreeding avoidance patterns ~~under natural~~
 223 ~~conditions, particularly in insects~~.

224 Here, we take up this challenge by studying a parasitoid wasp with a simple form of inbreeding
 225 depression, ~~both in the laboratory and in the field~~. Parasitoids are ~~haplodiploid~~, where ~~males develop~~
 226 ~~from unfertilized haploid eggs and females develop from fertilized diploid eggs~~. Many hymenopteran
 227 species ~~further have a single-locus Complementary Sex Determination (sl-CSD) mechanism, with which~~,
 228 sex is determined by both ploidy and heterozygosity at a unique sex determination locus (Cook, 1993;
 229 van Wilgenburg et al., 2006; Heimpel and de Boer, 2008; Asplen et al., 2009; Schmieder et al., 2012).
 230 Inbreeding depression arises for diploids that are homozygous at the sex locus, ~~because inbreeding~~
 231 ~~leads to a proportionally higher number of diploid males that are generally~~ unviable or sterile (Cook,
 232 1993; van Wilgenburg et al., 2006; Fauvergue et al., 2015). Whatever the diversity of sex alleles in the
 233 population, full sibs have 50% chance of sharing a common sex allele and, if they mate, half of their
 234 diploid offspring will develop into unfit males (Cook, 1993). ~~Assuming~~ females fertilize half of their
 235 eggs, sib-mating ~~thus~~ results in 12.5% fewer offspring, on average. sl-CSD is thus a form of inbreeding
 236 depression underlined by overdominance, with no deleterious alleles at the sex locus, but a strong
 237 heterozygous advantage (e.g., Charlesworth and Willis, 2009). The null fitness of diploid males should,
 238 ~~therefore, favour sib-mating avoidance in species with sl-CSD~~.

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277 The parasitoid wasp *Venturia canescens* Gravenhorst (Hymenoptera: Ichneumonidae) has a
 278 single locus complementary sex determination (Beukeboom, 2001) and inbreeding reduces the fitness
 279 of both males and females through the production of sterile diploid offspring. Inbreeding further has
 280 a negative impact on egg load and hatching rate (Vayssade et al., 2014; Chuine et al., 2015). In no-
 281 choice bioassays, mating success consistently decreased with increasing genetic relatedness between
 282 mates (Metzger et al., 2010a; Chuine, 2014). There is some evidence suggesting that females are the
 283 choosy sex, which makes sense in a species with a monandrous/polygenous mating system (Metzger
 284 et al., 2010a). When females were confronted with a choice between a brother and an unrelated male,
 285 however, females did not avoid inbreeding, potentially due to the small size of test tubes (i.e. where
 286 mixing of chemical signals may have occurred; Metzger et al., 2010a). In this study, we first tested the
 287 effect of male density and average relatedness on inbreeding avoidance in the laboratory (Metzger et
 288 al., 2010a). We expected sib-mating avoidance to decrease with decreasing density and increasing
 289 relatedness. We further implemented a population genetic approach based on microsatellite
 290 genotyping to assess inbreeding avoidance and underlying behaviours in field populations of *V.*
 291 *canescens*. For this, we sampled two natural populations of *V. canescens* and compared the observed
 292 rate of sib-mating to the probability of random encounters among genetically-related individuals.
 293 Under the hypothesis of sib-mating avoidance, we expected the rate of sib-mating to be lower than
 294 the probability that relatives encounter each other at random. We further used these field data to test
 295 whether sex-biased dispersal reduces encounters among sibs in the field. We show that sib-mating
 296 tolerance occurs in the wild and propose an evolutionary scenario under which sib tolerance may
 297 evolve without loss of the ability to discriminate kin. We discuss why our results differ from earlier
 298 findings of inbreeding avoidance in this species, as well as prior expectations about inbreeding
 299 depression in species with SI-CSD (van Wilgenburg et al., 2006).

300

301 **Materials and Methods**

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330 *Biological model*

331 *Venturia canescens* is a monandrous solitary endoparasitoid found in most circum-
 332 Mediterranean areas (Salt, 1976). Females lay eggs in a large range of hosts found on various fruits
 333 (Salt, 1976; Driessen and Bernstein, 1999). Knowledge of the mating system of *V. canescens* under
 334 natural conditions is limited, but can partly be inferred from [results of laboratory experiments](#). Females
 335 do not discriminate between host patches with more than four host larvae, suggesting that, in the
 336 field, [females](#) rarely encounter aggregated host (Driessen and Bernstein, 1999). It is, [therefore](#), unlikely
 337 that [V. canescens](#) experiences local mate competition, [where males remain at the natal patch to mate](#)
 338 [\(include ref Hamilton on LMC theory\)](#). Males search for females via a synergistic combination of volatile
 339 sex pheromones emitted by females and kairomones [released by](#) hosts (Metzger et al., 2010b). Both
 340 immature development and adult life lasts around three weeks (at 25°C), and females lay eggs during
 341 most of their adult life (Metzger et al., 2008), [leading](#) to overlapping generations. Depending on [the](#)
 342 [study considered](#), sex-ratio is [either](#) considered balanced (Beukeboom, 2001) or weakly biased toward
 343 females (Metzger et al., 2008). [Equal sex ratios are indeed expected when hosts do not aggregate](#)
 344 (Driessen and Bernstein, 1999), [local mate competition does not occur](#), and mate-finding [takes place](#)
 345 via volatile cues. The proportion of diploid males [further](#) corresponds to that expected [when sex ratios](#)
 346 [are equal](#) (Fauvergue et al., 2015). Field and laboratory studies [further revealed](#) that adult females are
 347 good dispersers, with a flight velocity estimated at 0.2 m.s⁻¹ (Schneider et al., 2002; Desouhant et al
 348 2003; Amat et al., 2012).

350 *Insect rearing*

351 [For laboratory experiments, we used a standardized laboratory rearing that had been](#)
 352 [established with about 60 females collected in the field near Valence \(same as the current location\),](#)
 353 [southern France, on several occasions during the summer of 2015. The host *E. kuehniella* was reared](#)
 354 [in the laboratory on organic wheat semolina medium for three weeks before being exposed to](#)

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371 [parasitoids \(eggs were obtained from the Biotop rearing facility located in Livron sur Drôme, France\).](#)
 372 [Parasitoid development took place in a controlled environment \(temperature: 24±1°C; relative](#)
 373 [humidity: 60±5%; photoperiod: LD 12:12\).](#)

374 [Experiments on female mate choice required related and unrelated males. We thus initiated](#)
 375 [families by collecting females randomly from the mass rearing \(from different rearing boxes\), and](#)
 376 [maintaining them individually in a circular box \(Ø: 8 cm, height: 3 cm\) with a meshed lid containing two](#)
 377 [males and about 30 three week-old host larvae on 1 cm of organic wheat semolina. The wasps were](#)
 378 [fed *ad libitum* with 50% water-diluted honey and allowed to lay eggs during their whole life.](#)
 379 [Approximately three weeks later, the parents were removed from the box if still alive and offspring](#)
 380 [collected at emergence, before reproduction. Daughters were kept individually in plastic vials, and](#)
 381 [males were kept with their brothers, both with access to a drop of water-diluted honey. All individuals](#)
 382 [used in the experiments were two days old.](#)

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384 *Insect sampling in the field*

385 [Adult *V. canescens* were captured from June to September 2014, during 10 non-consecutive](#)
 386 [days. Two field sites, located 300 km apart, were sampled: Valence \(N 44°58'21" E 4°55'39", composed](#)
 387 [of fruit trees in an orchard\) and Nice \(N 43°41'23" E 7°18'6", composed of carob trees in a semi-natural](#)
 388 [park\). We captured only females in Valence and wasps of both sexes in Nice. *Why did you only collect*](#)
 389 [females in Valence? I think you need to include some explanation here, because it would have been](#)
 390 [better if males were collected also from Valence.](#)

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391 [Two different types of traps were used: kairomone-baited traps for the capture of live females,](#)
 392 [and yellow sticky traps for males \(which were generally found dead\). Female traps constituted *of an*](#)
 393 [open cup containing host larvae, *Ephestia kuehniella* Zeller \(Lepidoptera: Pyralidae\), along with rearing](#)
 394 [medium \(semolina\). Saliva secreted by host larvae when feeding serves as a kairomone for female *V.*](#)

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401 *canescens* (Corbet, 1971). Traps were hung in trees (Metzger et al., 2008) and visited every 20 minutes
 402 to collect attracted females. For males, 125 mm × 200 mm yellow sticky cards were baited with extracts
 403 from females and hosts, a method that already proved successful previously (Collet et al., 2016). For
 404 each trap, a vial containing the extracts was attached to the centre of the card. For females, traps were
 405 hung within trees, and all *V. canescens* males that were found stuck on the traps collected and
 406 conserved individually in 96% ethanol to preserve DNA. We captured 77 females and 86 males in Nice
 407 and 78 females in Valence (see Results and Table 1).

408 Females collected in the field were brought back to the laboratory, maintained in a climatic
 409 chamber under constant conditions (temperature: 24±1°C, relative humidity: 60±10%; photoperiod:
 410 L:D 16:8) and fed 50% water-diluted honey. Each female was allowed to lay eggs individually in *E.*
 411 *kuehniella* hosts for three days. As we could not control for the age and egg-laying experience of
 412 captured females, the number of offspring was highly variable (1-10 daughters and 1-7 sons). As sexual
 413 (arrhenotokous) and asexual (thelytokous) strains coexist in *V. canescens* (Beukeboom et al., 1999;
 414 Schneider et al., 2002), we used the presence of males among offspring as evidence for arrhenotoky,
 415 and thelytokous individuals were discarded. Mothers and their offspring were then preserved
 416 individually in 96% ethanol.

418 Effect of male density and sib frequency on sib-mating avoidance in the laboratory

419 We aimed to untangle the effects of male density and the frequency of encounters with related
 420 males on female mate choice. To test this, we designed a factorial experiment with two densities of
 421 males (high density, noted *D*, with nine males, and low density, noted *d*, with three males) and two
 422 frequencies of related males (high frequency, noted *F*, with 2/3 of brothers, and low frequency, noted
 423 *f*, with 1/3 of brothers). Males were marked according to kinship (brother vs unrelated) with a drop of
 424 paint applied on their thorax 24h before being tested. The two colours used do not modify behaviours
 425 (Desouhant et al., 2003), but were nonetheless alternated randomly across trials. Each trial took place

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442 as follows: a virgin female was released in a mesh cage (30 × 30 × 30cm) containing males at a
 443 [temperature of 25°C](#), [a relative humidity of 65%](#) and [under](#) constant luminosity. Courtship and mating
 444 behaviours were monitored for 30 min or until a mating had occur between 10 am and 4 pm ([How](#)
 445 [were these behaviors recorded? Was a computer program used? If yes, then mention which one, if no,](#)
 446 [then explain how the observer kept track of behaviors](#)). We recorded mating latency, copulation
 447 duration, relatedness of the successful male, and courtship effort (*i.e.* the duration of each male
 448 courtship behaviour) (van Santen and Schneider 2002). For each courtship [sequence](#), we identified the
 449 relatedness (colour) of the [courter](#); hence all courtships were counted independently of mating
 450 success. Twenty trials were performed per factor level, except for *d-F* (18 replicates).

451 We analysed the proportion of sib-mating with a [Generalized Estimating Equation –](#)
 452 [Generalized Linear Model](#) (GEE-GLM; Liang and Zeger, 1986, GEEPACK package in R software)
 453 implemented with a binomial [error](#) distribution, a logit link function, an exchangeable correlation
 454 structure, and a fixed scale. The model was fitted with mate relatedness as a response variable
 455 (binomial: 0 if the female mated with her brother, 1 if she mated with an unrelated male), density and
 456 frequency of brothers as main effects and in interaction as explanatory variables, and families as a
 457 random factor (*i.e.* clusters, Højsgaard et al., 2006). We added an offset term to take into account the
 458 theoretical mating proportion with related males (*i.e.* 1/3 in low frequency *f* and 2/3 in high frequency
 459 *F*). We further tested the effects of density and proportion of brothers on male courtship effort and
 460 latency before copulation. For courtship effort, we implemented a GLM based on a binomial
 461 distribution and a logit link function, with the percentage of courtships done by brothers as a response
 462 variable and the density and proportion of brothers as explanatory variables. For latency before
 463 copulation, we performed a Cox model with the same explanatory variables as fixed effects and family
 464 as a random effect (COXME package, Therneau, 2015 in R software). We also included the number and
 465 [duration](#) of courtship (from a related or unrelated male) as a time-dependent variable. If females
 466 assess relatedness with males during courtship, an increasing number of encounters with related
 467 males could lead the female to decrease [her](#) selectivity and, as a consequence, the latency before

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487 mating. Alternatively, an increasing number of encounters with unrelated males should not affect her
 488 selectivity and therefore her latency to mate.

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490 Genotyping

491 Genotyping was performed at 18 microsatellite loci developed by Collet et al. (2016) and
 492 Mateo Leach et al. (2012) (See SEM-A for details on the genotyping and SEM-D for details on the
 493 number of microsatellites used). We genotyped all field-captured males and females (hereafter
 494 referred to as wild females) and up to three daughters per female (see SEM-B for a justification of the
 495 number of offspring genotyped). When fewer than three daughters emerged (ca. 34% of which 40%
 496 produced only one daughter) all were genotyped.

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497 We estimated polymorphism and linkage disequilibrium at each microsatellite locus for the
 498 wild females of Nice and Valence with the GENEPOP software version 4.3 (Raymond and Rousset, 1995).
 499 The frequency of null alleles was estimated with FREE NA software (Chapuis and Estoup, 2007).
 500 Population differentiation was estimated based on Wright's F_{st} estimator F_{st} with the FSTAT software
 501 version 2.9.3 (Goudet, 1995). Effective population sizes were estimated using the full likelihood
 502 method, assuming random mating, developed by Wang (2009), and implemented in the software
 503 COLONY (Jones and Wang, 2010). As effective population sizes were small (< 80), we did not check for
 504 Hardy-Weinberg equilibrium. We also determined the number of diploid males as a proxy for
 505 consanguinity and the number of alleles at the *csd* locus. A male was considered diploid if at least one
 506 microsatellite locus was heterozygous, haploid otherwise (Collet et al., 2016).

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508 Inference of relatedness among mates

- 509 • Male genotypes

519 We inferred the genotype of wild females' mates based on the genotypes of wild females and
520 their daughters. For this, we used the software COLONY, which reconstructs families based on
521 haplodiploid genotypes (Jones and Wang, 2010). When alternative genotypes were proposed for a
522 given male, we selected the one that was compatible with the genotype of the daughter and the
523 mother. When several alternatives were possible (e.g. if mother and daughters were heterozygous for
524 the same alleles at a given locus), it was impossible to assign the right allele to the father with certitude.
525 In this case, we assigned the allele with the highest probability of occurrence (the probability is given
526 by COLONY. We successfully inferred the genotype of 51 mates for the 54 wild females captured (see
527 Results and Table 1).

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528 • Relatedness between mates

529 To assess if *V. canescens* females avoid mating with relatives in the field, we compared the
530 observed number of wild females mated with a related male to the number expected under the
531 assumption of random mating. For this, we determined a "relatedness threshold" above which
532 individuals were considered genetically related. To determine this threshold, we simulated
533 populations with similar allele frequencies at microsatellite loci to that of natural populations, and a
534 balanced sex ratio (see SEM-C for details on simulations). Simulations allowed to keep track of
535 pedigrees, yielding a perfect knowledge of kinship for all potential mates, which we classified in three
536 categories: full-sibs, half-sibs, or unrelated. Using microsatellite genotypes from the same simulated
537 dataset, we estimated the relatedness coefficient (r) for all female-male pairs (TrioML coefficient,
538 software COANCESTRY; Wang, 2011). We then used these estimated relatedness coefficients with
539 different relatedness thresholds to assign each dyad to related vs unrelated categories (SEM-C). By
540 comparing these posterior assignments to the pedigrees known a priori, we estimated the relatedness
541 threshold that minimized the number of wrong assignments (*i.e.* unrelated pairs assigned related
542 based on the threshold, or vice-versa) under the random encounter hypothesis. We found $r_{crit} = 0.16$
543 (SEM-D, Fig. S2). Logically, this threshold is lower than the theoretical relatedness between a brother

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548 and a sister ($r_{\text{sister-brother}} = 0.25$; $r_{\text{brother-sister}} = 0.5$) and in the interval of relatedness for 1st degree cousins
 549 ($r = 0.125$ or $r = 0.1875$ depending on the cross). With this threshold, we expect to wrongly assign
 550 11.4% of the relationships.

551 We applied this $r_{\text{crit}} = 0.16$ threshold to field data. First, we estimated the number of wild
 552 females that had been inseminated by a related male. Second, we generated a theoretical distribution
 553 of the number of couples with $r \geq 0.16$ under the null hypothesis of random mating. For this, each
 554 of the 51 wild females from Nice, for which a mate genotype had been successfully inferred, was paired
 555 randomly with one of the 79 genotyped males, and we counted the number of pairs with $r \geq 0.16$. This
 556 calculation was repeated 1000 times to produce the null distribution. We finally compared the
 557 observed number of related pairs with the null distribution via a permutation test implemented in the
 558 function `AS.RANDTEST` from the `ADE4` package in R software version 3.2.3 (Chessel et al., 2004; R Core
 559 Team, 2015). We also estimated the probability that a female encountered a related male in the field
 560 the day of her capture. Indeed, partners present on the same day in the same area have a high chance
 561 of encountering each other and mate due to the high dispersal capacity of this species.

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563 *Sex-biased dispersal in the field*

564 Sex-biased dispersal can decrease the burden of inbreeding depression by reducing encounters
 565 and subsequent mating among sibs. We thus assessed the dispersal ability of males and females from
 566 our genotyping data in the population of Nice, where two trapping locations were distinguishable
 567 (Figure 1). The first patch contained a group of 21 trees and the second patch 15 trees. Trees were
 568 located a few meters from one another within patches, and the two patches were about 200 m away.

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569 Six sampled trees further apart were not included in this scheme (black dots in Figure 1).

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570 We estimated the relatedness between individuals of each sex within and between patches
 571 using COANCESTRY software (Wang, 2011). Sex-biased dispersal should result in higher coefficients of

577 relatedness within patches than between patches for the sex dispersing at smaller distances (Prugnolle
 578 and de Meeus, 2002). Coefficients of relatedness inter-patch and intra-patch were compared via a
 579 Kruskal-Wallis test (with patch as an explanatory factor) for each sex separately. When significant,
 580 post-hoc Mann-Whitney's *U*-tests (with Bonferroni corrections) between the three modalities (i.e.
 581 relatedness within patch 1, relatedness within patch 2, and relatedness between patches) were
 582 performed.

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583

584 Results

585 *Effect of male density and sib frequency on sib-mating avoidance in the laboratory*

586 Mating success was higher at high male density (*D*; 81.6%, 40 matings/49 trials) than at low
 587 density (*d*; 44.1%; 38/86) (Table 3; proportion test: $\chi^2 = 16.44$, $df = 1$, $p = 5.02 \cdot 10^{-5}$). The observed
 588 proportion of sib-mating did not depart from that expected under random encounters (GEE-GLM
 589 model, *p*-value of the intercept = 0.3). This was confirmed by a positive relationship between the
 590 proportion of related males and the probability of sib-mating (Wald statistic table, $\chi^2 = 15.36$, $df = 1$,
 591 $p = 8.9 \cdot 10^{-5}$). Male density had no effect on mate choice, neither as a main effect ($\chi^2 = 0.92$, $df = 1$,
 592 $p = 0.34$) nor in interaction with the proportion of related males ($\chi^2 = 0$, $df = 1$, $p = 1$). Males did not
 593 differ in their courtship effort, as the proportion of courtships observed for unrelated males and
 594 brothers did not depart from the expected values (GLM model, *p*-value of the Intercept = 0.16): for
 595 example, in the *D-F* modality with 6 brothers and 3 unrelated males, we expected that 2/3 of the
 596 courtships would be done by brothers (i.e. 13.3 on 20 matings) and observed 12 sib-matings. Time
 597 before copulation decreased with increasing male density and increasing number of sib rejections
 598 (Table 4), but it was not affected by the proportion of related males or by the number of rejections of
 599 unrelated males (Table 4).

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600 *Genotyping*

607 A total of 241 wasps were captured in Valence and Nice. In Valence, 78 wild females were
 608 captured (Table 1) of which 35 produced a total of 86 daughters (Table 1; 5.1 ± 0.4 offspring, including
 609 2.4 ± 0.2 daughters per female). In Nice, 86 males and 77 females were captured, of which 54 produced
 610 140 daughters (Table 1; 4.8 ± 0.3 offspring, including 2.6 ± 0.1 daughters per female). We genotyped
 611 467 of these individuals at 18 microsatellite markers. We obtained the genotype at all 18 microsatellite
 612 loci for 402 individuals, at 15-17 loci for 50 individuals, 1-10 loci for 11 individuals, and no amplification
 613 was obtained for 4 individuals (Table 1). Only individuals with successful amplification at >14 loci were
 614 included in the dataset, which represented 452 individuals (96.8%).

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615 No linkage disequilibrium was detected and null allele frequency was always lower than 6.5%
 616 in the two populations. We found no genetic differentiation between the two populations ($F_{st} = 0.01$).
 617 We, nonetheless, treated the two populations separately because the probability that a male captured
 618 in one population had inseminated a female captured 300 km apart is negligible. Effective population
 619 sizes were estimated to be 79 for Nice (IC 95% [44; 82] *What are these values? It looks like numbered*
 620 *references, please check*) and 51 for Valence (IC 95% [26; 56]). These values are low and not
 621 significantly different.

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623 *Sib-mating avoidance in the field? Relatedness of actual versus potential mates*

624 In Nice, the mean coefficient of relatedness between wild males and females was
 625 $r = 0.047 \pm 0.001$. We estimated that $11\% \pm 0.79\%$ (IC 95%) of the relatedness coefficients between
 626 these individuals were higher than the threshold $r_{crit} = 0.16$ (671 comparisons over 6083, from 79 males
 627 \times 77 females); the corresponding pairs were thus considered genetically related. The average
 628 probability that a female encountered a related male in the field on the day of capture was $12.0\% \pm 4.9$
 629 (Fig. 2).

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636 Thirty-five and 54 wild females from Valence and Nice, respectively, produced at least one
 637 daughter which allowed to infer the genotype of their mate (Table 1). The mean relatedness between
 638 these inferred pairs was 0.050 ± 0.009 in Nice and 0.044 ± 0.013 in Valence. Assuming $r_{crit} = 0.16$, we
 639 found six and four genetically related pairs in Nice and Valence, respectively, that is, $11.1\% \pm 8.4\%$
 640 [\(What is this values? Standard deviation? Please explain throughout the manuscript.\)](#) and
 641 $11.4\% \pm 10.5\%$ of all pairs. For the population of Nice, this observed number of related pairs did not
 642 differ from the number expected under the assumption of random mating (1000 permutations; mean
 643 number of related pairs expected from random mating = 5.264; number observed = 6, $p = 0.822$; Fig.
 644 3).

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646 *Proportion of diploid males and number of CSD alleles*

647 [We](#) assessed the cost of inbreeding by estimating the number of diploid males in the
 648 population of Nice (no males [were](#) captured in Valence). In species with sl-CSD, diploid males result
 649 from matched matings (parents sharing a common allele at the *csd* locus), and the probability of
 650 matched mating in a panmictic population is $2/k$, where k is the effective number of *csd* alleles. The
 651 number of alleles at the *csd* locus k could be estimated from the proportion of diploid males among
 652 the males m and the proportion fertilizations s (see SEM-E for the calculation). As [previous findings](#)
 653 [suggest](#) a balanced sex-ratio [\(include references here\)](#), we set $s=0.5$. We estimated the number of *csd*
 654 alleles in the population of Nice, where random matings [is expected](#) (see previous section). Six diploid
 655 males were found among the 79 males captured ($m=7.6\% \pm 5.8\%$, IC 95%) yielding an estimation of 8.8
 656 alleles at the *csd* locus and a probability $2/k = 0.23$ of matched matings.

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658 *Sex-biased dispersal in the field*

668 During the field experiment, 50 females and 47 males were genotyped in patch 1, 18 females
 669 and 22 males in patch 2. Genetic relatedness was estimated among females and among males within
 670 patches (referred to as 1-1 for intra-patch 1 and 2-2 for intra-patch 2), as well as between patches
 671 (referred to as 1-2) (Table 2). Mean relatedness differed within and between patches for females
 672 (Kruskal-Wallis test: $\chi^2 = 14.88$, $df = 2$, $p < 1.10^{-4}$), but not for males (Kruskal-Wallis test: $\chi^2 = 1.72$, $df =$
 673 2 , $p = 0.424$). For females, relatedness was similar within each of two patches (1-1 and 2-2; Mann-
 674 Whitney test: $U = 80550$, $p = 0.520$; Table 2) and higher than between patches (1-1 / 1-2 comparison,
 675 Mann-Whitney test: $U = 56724$, $p < 4.10^{-4}$; 2-2 / 1-2 comparison, Mann-Whitney test: $U = 65356$, $p =$
 676 0.012). Sex-biased dispersal, therefore, occurs in *V. canescens*, with males dispersing more than
 677 females.

679 Discussion

680 Sib-mating avoidance in *Venturia canescens* was previously observed in no-choice behavioural
 681 experiments conducted under laboratory conditions (Metzger et al., 2010a, Chuine, 2014). We show
 682 here that when females can choose between related and unrelated males, sib-mating does occur. The
 683 density of males and the proportion of related individuals in the surroundings do not change the
 684 pattern of inbreeding avoidance, suggesting that relatedness is not a cue for mate choice. Females
 685 nonetheless appear to discriminate sibs from non-sibs, because mating latency decreases after
 686 rejection of a sib. The sib-mating tolerance that we observed in the laboratory was confirmed by our
 687 genetic approach with field populations. We showed that the observed frequency of sib-mating
 688 corresponds to the probability of sib-mating expected when sib encounters occur at random. Our field
 689 data further suggest there is sex-biased dispersal, but without yielding passive sib-mating avoidance.
 690 We will next propose an evolutionary scenario to explain the maintenance of kin recognition, and its
 691 apparent absence of use in the mate choice context, in the laboratory and in the field.

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714 In laboratory cages, the proportion of sib-mating perfectly matched the proportion of sibs
 715 present. Mating rates between relatives, be they inferred from genetic analyses or from a simulation
 716 model assuming random encounters, were both equal to 11%. These two results are in line with
 717 expectations in the absence of sib-mating avoidance in *V. canescens*, and brings into question past
 718 evidence of kin recognition and avoidance in the context both of host parasitization (Bernstein and
 719 Driessen, 1996; Marris et al., 1996) and mate choice (Metzger et al., 2010a; Chuine et al., 2015). In
 720 particular, no-choice bioassays previously revealed that female *V. canescens* mated twice more often
 721 with unrelated males than with their brothers, and were therefore capable of sib-mating avoidance
 722 (Metzger et al. 2010a). In the presence of volatiles from brothers, females also discriminated against
 723 unrelated males, suggesting that mate-choice could be underlined by volatile cues (Metzger et al.,
 724 2010a; Chuine et al., 2015). Our laboratory cage experiment also suggests that females are able to
 725 recognize kin and modify a component of their mating behaviour (latency before copulation), but
 726 without sib-mating avoidance.

727 The lack of evidence for kin discrimination in our field study could result from a
 728 misclassification of cousins or more distantly related individuals as full sibs. When genetic relatedness
 729 decreases to that of cousins ($r \leq 0.1875$), sib-mating avoidance in *V. canescens* declines steeply (Chuine,
 730 2014). Misclassifying cousins as brothers would thus, unsurprisingly, yield an absence of sib-mating
 731 avoidance. This is, however, unlikely. We classified pairs as sibs versus non-sibs based on a threshold,
 732 $r_{crit} = 0.16$, that proved to minimize the probability of misclassification (about 10% of errors). Moreover,
 733 increasing this threshold to further restrict the definition of kinship did not change our conclusions,
 734 that is, the observed number of related pairs was always similar to that expected under random mating,
 735 ($r_{crit} = 0.2$: 1000 permutations, 2 observed related pairs, 1.88 expected related pairs, $p = 1$; $r_{crit} = 0.25$:
 736 1000 permutations, 0 observed related pairs, 0.999 expected related pairs, $p = 0.637$). The hypothesis
 737 that poor kinship assignation yielded spurious results thus falls short of argument, and we conclude
 738 that the lack of evidence for sib-mating avoidance truly reflects a lack of sib-mating avoidance, also
 739 called sib-mating tolerance.

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765 Sex-biased dispersal can also shape the pattern of inbreeding avoidance in the field (Gandon,
 766 1999; Roze and Rousset, 2005). In *V. canescens* we found a slight male-biased dispersal at a local scale
 767 (Table 2). However, in the population of Nice, the relatedness values between and within patches
 768 (between which dispersal occurs) in both sexes was low (between 0.042 and 0.065, Table2). If male-
 769 biased dispersal indeed affected the structure of this population and encounter rates between
 770 relatives, it should be a very weak effect. You need to develop this paragraph further. Why not discuss
 771 some of the work that has been done on sex-biased dispersal in the field in other animals? And then
 772 conclude that sex-biased dispersal should only have a weak effect in your system.

773 Density-dependent female choosiness in *V. canescens*, as shown in other species (Kokko and
 774 Rankin, 2006; Duthie and Reid, 2016), could also explain the absence of sib-mating avoidance.
 775 Avoidance of sibs would arise in dense or aggregated populations with high mate encounter rates, but
 776 not in scarce populations where the cost of discrimination – remaining virgin – is higher. This was
 777 shown in a small isolated population of moose, where females accepted mates with higher relatedness
 778 during years when males were less available (Herfindal et al., 2014). A similar reasoning may also apply
 779 to highly inbred populations, where sib-mating avoidance would result in a high probability of not
 780 mating at all, a penalty that could exceed the costs of inbreeding depression (Kokko and Ots, 2006). In
 781 *V. canescens*, population density indeed affects mating success but not sib-mating probability.
 782 Moreover, the proportion of related males that we tested in the laboratory were respectively twice
 783 and four times higher than those found in the field, and still, no sib-mating avoidance was observed.
 784 In the field, the distribution of the sib-encounter probability is skewed towards zero, leading to more
 785 than half of *V. canescens* females having no chance to encounter a related male on a particular day,
 786 possibly because of the low number of individuals in the field (Fig. 2). The average probability for a
 787 female to encounter a relative is nonetheless within the same range as actual sib-matings (12%; Fig.
 788 2). Kin recognition mechanisms could then have persisted, but not sib-mating avoidance. We therefore
 789 conclude that *V. canescens* does not avoid sib-mating, whatever the density of males and level of
 790 inbreeding.

Supprimé: The second hypothesis is that we could face the ghost of past selection for sib-mating avoidance. If the cost of inbreeding was higher in the evolutionary history of *V. canescens*, behaviours allowing kin recognition and reducing the risk of inbreeding could have been selected for. - ... [3]

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816 Female kin discrimination against related males could also decay over time. Encountering a
 817 succession of low-quality males (*i.e.* genetically-related in our study) could decrease female
 818 choosiness, similar to what was demonstrated for a monoandrous wolf spider (Stoffer and Uetz, 2016).
 819 We have shown in our laboratory experiment that even if females did not avoid sib-mating, the latency
 820 before copulation decreased significantly after having rejected a brother. This result confirms that *V.*
 821 *canescens* is capable of discriminating relatives in a context of mating (see also Metzger et al., 2010a;
 822 Chuine, 2014; Chuine et al., 2015) and suggests that the presence of relatives could affect mating by
 823 decreasing the latency before copulation, as expected under this hypothesis. More experiments are,
 824 however, needed to firmly test the evolution of female choosiness over time.

825 Tolerance to sib-mating, such as that observed in *Venturia canescens*, is often explained by low
 826 inbreeding depression or high costs of outbreeding (Kokko and Ots, 2006; Puurtinen, 2011). We found
 827 a low effective population size at both sample sites ($N_e = 51$ in Valence, $N_e = 79$ in Nice), within the
 828 same range as threatened bumblebees (Ellis et al., 2006) or Hine's emerald dragonflies (Monroe and
 829 Britten, 2015). *V. canescens* could be prone to loss of genetic diversity, possibly amplified by inbreeding
 830 tolerance, which would in turn increase inbreeding load. Alleles at the *csd* locus, the major mechanism
 831 of inbreeding depression in *V. canescens*, are, however, under strong balancing selection and are thus
 832 expected to overcome genetic impoverishment, similar to observations in populations of the Asiatic
 833 honeybee (Gloag et al., 2016). Moreover, inbreeding in *V. canescens* only has a moderate negative
 834 impact on egg load and hatching rate, and no effect on other fitness components, such as mating
 835 probability or body size (Vayssade et al., 2014).

836 Despite common beliefs, the genetic load resulting from sl-CSD might not be dramatic in
 837 *Venturia canescens*. In the population of Nice, we estimated 8.8 alleles at the *csd* locus, which is in the
 838 range of what was previously found for other species in the order Hymenoptera (8-86 alleles, mostly
 839 between 9 to 20; Cook and Crozier, 1995). Under random mating, this yields a probability of matched-
 840 matings of 23% (2/8.8). In case of brother-sister mating, the probability of matched matings increases

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865 to 50%. Assuming that females fertilize half of their eggs so that matched matings results in 25% diploid
 866 males, and that diploid males are sterile (Fauvergue et al. 2015), the expected reduction in the number
 867 of fertile offspring under random mating would be 5.8% (0.23×0.25). If sib-mating was perfectly
 868 avoided, the expected reduction in the number of fertile offspring would be 4.8% (0.19×0.25 , see
 869 SEM-E for the calculation). Sib-mating avoidance would thus result in about 1% more fertile offspring
 870 in the populations we studied, a fitness gain that may appear insignificant. The relatively low
 871 probability of matched matings and the consequent benign inbreeding load may thus be insufficient
 872 to select for sib-mating avoidance. *V. canescens* may thus be an example of a species with inbreeding
 873 tolerance (Waser et al., 1986; Kokko and Ots, 2006).

874 Discrepancies between sib-mating avoidance revealed in previous research and our finding of
 875 inbreeding tolerance could highlight the evolution of kin-recognition mechanisms for purposes other
 876 than sib-mating avoidance. This would have produced artefactual behaviours when observed in small
 877 arenas (Metzger et al., 2010a), but vanishing in larger cages and in the field. Evidence for active kin
 878 recognition is pervasive in various ecological contexts such as territory defence, reciprocal altruism, or
 879 dominance hierarchies (Mateo, 2004). In *V. canescens*, the evolution of kin recognition for the
 880 avoidance of superparasitism could explain the maintenance of kin recognition without sib-mating
 881 avoidance. Indeed, females are able to discriminate the self, their relatives and their conspecifics when
 882 laying eggs, and avoid already parasitized hosts. As only a single offspring can develop in a host,
 883 superparasitism has a high cost for female fitness. Consistently, the intensity of superparasitism
 884 avoidance increases with genetic relatedness to the first laying female (Hubbard et al., 1987; Marris et
 885 al., 1996; Amat et al., 2009). If kin recognition evolved in this context, kin discrimination in a situation
 886 of mate choice could be a by-product of this primary selection on kin recognition. We could therefore
 887 expect a relatively general molecular pathway, which would have been expressed in a small-scale
 888 mating bioassay: kin recognition during mating is probably mediated by semiochemicals from the
 889 insect cuticle (cuticular hydrocarbons, Metzger et al., 2010a; Chuine, 2014) and discrimination against
 890 superparasitized larvae is driven by weak volatile hydrocarbon signals left by females during

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900 oviposition (Fisher, 1961; Harrison et al., 1985). These mechanistic similarities point towards a unique
 901 pathway for kin recognition. Superparasitism avoidance could act as a strong selective pressure for the
 902 maintenance of kin recognition, because the cost of superparasitism is positively correlated with the
 903 relatedness among competing females. Further experiments are needed to test the idea that sib-
 904 mating avoidance observed under laboratory conditions is a by-product of cognitive abilities that
 905 evolved in the context of intraspecific competition.

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Mis en forme: Anglais (E.U.)

Supprimé: Does Kin Recognition and Sib-Mating Avoidance Limit the Risk of Genetic Incompatibility in a Parasitic Wasp?

Mis en forme: Néerlandais (officiel)

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1139 Authors' contributions

- 1140 M.C., I.A., X.F., L.M. and E.D. designed the experiment and M.C., S.S and A.A collected the data. M.C.,
 1141 I.A., L.M. and E.D. designed and interpreted the analyses. M.C. conducted the analyses and assembled
 1142 a first draft. M.C., I.A., X.F., L.M. and E.D. all contributed to revisions.

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1148

1149 **Table 1** – Number of adult *V. canescens* captured from wild populations in Nice and Valence.

1150 Pop = population; Number of mothers = number of wild females captured in the field that produced
 1151 at least one daughter; F = female, M = male.

1152

Pop	Sex	Wild individuals	Wild individuals successfully genotyped	Number of mothers	Total number of daughters	Daughters successfully genotyped
Nice	F	77	77 (100%)	54	140	134 (96%)
	M	86	79 (92%)	-	-	-
Valence	F	78	77 (99%)	35	86	85 (99%)

1153

1154

1155 **Table 2** – Mean relatedness r (\pm SE) among females and among males of *V. canescens*, either within
 1156 patches (1-1 or 2-2) or between patches (1-2) of host plants (carob trees) in the population of Nice.

1157 F = female, M = male.

Sex	Patch	Mean r (\pm SE)
F	1-1	0.065 \pm 0.002
	2-2	0.060 \pm 0.006
	1-2	0.050 \pm 0.002
M	1-1	0.042 \pm 0.002
	2-2	0.048 \pm 0.006
	1-2	0.043 \pm 0.003

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1164 **Table 3** - Effect of male density and sib frequency on sib-mating avoidance in *V. canescens*. Two
 1165 densities of males (*D*: high density = 9 males, and *d*: low density = 3 males) and two frequencies of
 1166 brothers (*F*: high frequency = 2/3 brothers and *f*: low frequency = 1/3 brothers) were manipulated in a
 1167 cage experiment. Presented are the number of successful matings for each factor level (20, except for
 1168 the *d*-*F* modality that contained only 18 replicates).
 1169

		D	d
F	Brother	12	12
	Unrelated	8	6
	Total	20/23 trials	18/44 trials
f	Brother	6	6
	Unrelated	14	14
	Total	20/26 trials	20/42 trials

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1175 **Table 4** – Effect of male density, sib frequency (and their interaction) and the number of male
 1176 rejections on mating latency in *V. canescens*. We analysed the deviance table of the mixed-effect Cox
 1177 model with the female family as a random effect. [Significant effects are in bold.](#)
 1178

	Log likelihood	χ^2	<i>df</i>	<i>p</i> -value
NULL	-351.51			
Male density	-338.44	26.14	1	3.17 .10⁻⁷
Sib frequency	-337.57	1.74	1	0.19
Rejection of sib	-332.45	10.25	1	1.36 .10⁻³
Rejection of unrelated	-331.46	1.97	1	0.16
Density × sib frequency	-331.46	0.01	1	0.92

Mis en forme: Police :Gras

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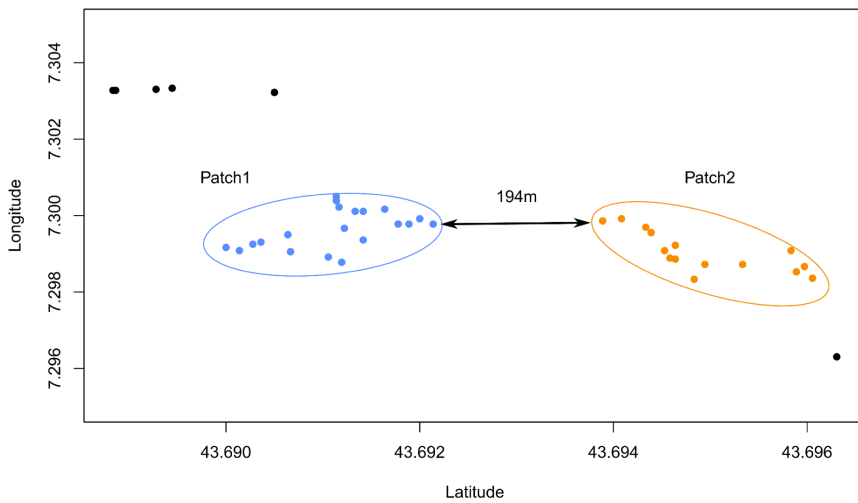


Figure 1 – Location of carob trees at the Nice field site where *V. canescens* were captured during the summer of 2014. Each dot represents a tree. Axes are GPS coordinates in decimal degrees. Blue dots represent trees of “Patch 1” and orange dots represent trees of “Patch 2” (see also Table 2). Six trees (black dots) were not included because they fell outside the main patch areas.

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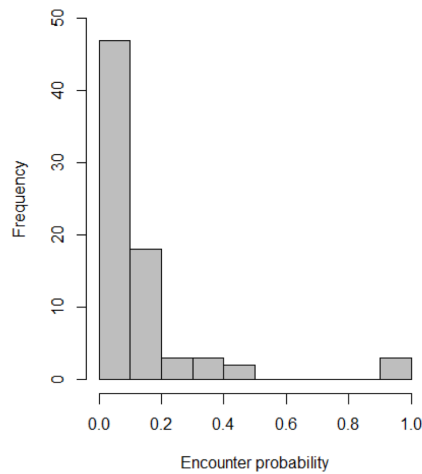


Figure 2 – Distribution of the probability that a female *V. canescens* encountered a genetically related male during the day of capture in the population from Nice. For each female caught, we computed her relatedness with all males captured the same day. We then counted the number of relationships with $r \geq 0.16$ and divided this number by the total number of relatedness computed (*i.e.* the number of males captured the same day).

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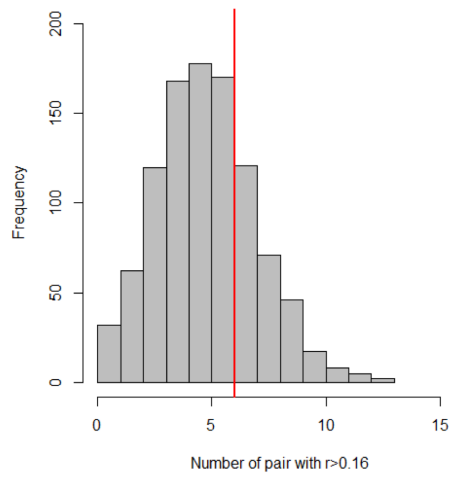


Figure 3 – Theoretical distribution of the number of crosses between genetically related *V. canescens* males and females under the assumption of random mating. The theoretical distribution was built using random sampling of a mate for each of the 51 females from the Nice population, estimating r for each pair and counting those with $r > 0.16$. Sampling was reiterated 1000 times. The red line represents the observed number of pairs with a $r > 0.16$.

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Insect rearing

The wild females trapped were brought back to the laboratory where they were handled in a climatic chamber under constant conditions (24±1°C, 60±10% RH, LD 16:8) and fed with 50% water-diluted honey. Each female was allowed to lay eggs individually in E. kuehniella hosts for three days. As we could not control for the age and previous egg-laying experience of captured females, the number of offspring was highly variable (1-10 daughters and 1-7 sons). As sexual (arrhenotokous) and asexual (thelytokous) strains coexist in V. canescens (Beukeboom et al., 1999; Schneider et al., 2002), we used the presence of males among offspring as evidence for arrhenotoky, and thelytokous individuals were discarded. Mothers and their offspring were then preserved individually in 96% ethanol. Insect rearing

For laboratory experiments, we used a standardized laboratory rearing. The wasp culture had been established with about 60 females collected in the field near Valence (same location as the field capture, N 44°58'21" E 4°55'39", INRA, Gotheron), southern France, on several occasions during the summer 2015. *Ephestia kuehniella* was reared in the laboratory on organic wheat semolina medium for three weeks before being exposed to parasitoids (eggs were obtained from the Biotop rearing facility located in Livron sur Drôme, France). Parasitoid development took place in a controlled environment (24±1°C; 60±5% RH; LD regime 12 h:12 h).

Experiments on female mate choice required related and unrelated males. We thus initiated families by collecting females randomly from the mass rearing (from different rearing boxes), and maintaining them individually in a little circular box (Ø: 8 cm, height: 3 cm) with meshed lid with two males and about 30 three-weeks host larvae on 1 cm of organic wheat semolina. The wasps were fed *ad libitum* with water-diluted honey (50-50) and allowed to lay eggs during their whole life. About three weeks after, the parents were removed from the box if still alive, and their offspring were

collected at emergence, before reproduction. Females (the daughters) were kept individually in plastic vials with a drop of water-diluted honey, and males were kept with their brothers and fed similarly. All individuals used in the experiments were two-days old.

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The second hypothesis is that we could face the ghost of past selection for sib-mating avoidance. If the cost of inbreeding was higher in the evolutionary history of *V. canescens*, behaviours allowing kin recognition and reducing the risk of inbreeding could have been selected for.

A third hypothesis is that s