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Establishment of F₁ hybrid mortality in real time

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Abstract

Background: Measuring the evolutionary rate of reproductive isolation is essential to understanding how new species form. Tempo calculations typically rely on fossil records, geological events, and molecular evolution analyses. The speed at which genetically-based hybrid mortality arises, or the “incompatibility clock”, is estimated to be millions of years in various diploid organisms and is poorly understood in general. Owing to these extended timeframes, seldom do biologists observe the evolution of hybrid mortality in real time.

Results: Here we report the very recent spread and fixation of complete asymmetric F₁ hybrid mortality within eight years of laboratory maintenance in the insect model *Nasonia*. The asymmetric interspecific hybrid mortality evolved in an isogenic stock line of *N. longicornis* and occurs in crosses to *N. vitripennis* males. The resulting diploid hybrids exhibit complete failure in dorsal closure during embryogenesis.

Conclusion: These results comprise a unique case whereby a strong asymmetrical isolation barrier evolved in real time. The spread of this reproductive isolation barrier notably occurred in a small laboratory stock subject to recurrent bottlenecks.

Keywords: *Nasonia*, Hybrid incompatibility, Development, Reproductive isolation, Speciation

Background

Given the importance of determining the patterns that affect the tempo of speciation, renewed emphasis has been placed on understanding how fast reproductive isolation barriers evolve during the speciation process. In the laboratory, complete F₁ hybrid mortality has never been documented to evolve *de novo*, while there are a few cases of incomplete premating isolation [1–4] and a case of hybrid reproduction defects in experimentally evolved yeast [5]. In mammals and birds, the evolution of strong hybrid mortality takes, on average, four million and 21 million years [6–8], respectively. In frogs, estimates suggest the minimum age for total hybrid inviability to become fixed is 1.5 million years [9, 10]. However, incomplete reproductive isolation and/or segregating variation for hybrid incompatibles can occur within various species, such as *Arabidopsis* [11] and *Tribolium* [12]. Intra-specific variation in hybrid incompatibilities indicates that there is segregating variation for hybrid incompatibility

alleles, but it does not inform when the incompatibility factors arose or spread *per se*. Taken together, the estimated timespans for severe hybrid mortality to fix in natural populations suggests that its emergence is often slow and/or restricted to large populations.

The genus *Nasonia* includes four closely related species of parasitic wasps that diverged between 0.3 to 1.0 million years ago [13]. These species include *N. vitripennis*, *N. longicornis*, *N. giraulti*, and *N. oneida*. The latter three species evolved sympatrically within the geographic range of *N. vitripennis*. The sister species, *N. giraulti* and *N. oneida*, occur sympatrically and share a distribution in the North Eastern temperate zone of North America, while *N. longicornis* lives in allopatry from *N. giraulti* and *N. oneida* on the western side of the continent. All four species are commonly used in laboratory studies of interspecific differences in their genetics, phenotypes, and microbial symbionts (reviewed in [14]).

Multiple *Wolbachia* infections exist within *Nasonia* and cause cytoplasmic incompatibility between the species [15, 16]. When reared under the same conditions in and before 2000 and cured of their *Wolbachia* infections,

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N. longicornis and *N. vitripennis* produced similar numbers of viable F₁ hybrid offspring (90–100%) in comparison to parental controls [16] (Fig. 1a). Indeed, all intraspecific crosses in the *Nasonia* genus readily produce F₁ hybrids in the absence of *Wolbachia* [15–17]. However, hybrid breakdown is commonly observed in the haploid F₂ hybrid males wherein cytonuclear incompatibilities and host-microbiota interactions cause hybrid larval mortality [18–21].

Recently, while attempting to perform experiments on F₂ hybrid breakdown between laboratory strains of *Nasonia longicornis* and *Nasonia vitripennis*, we discovered a novel F₁ hybrid lethality between these species (Fig. 1b). It is asymmetric, complete, and occurs in the cross between *N. vitripennis* males and *N. longicornis* females. This new and unexpected F₁ hybrid malady affords an opportunity to time the evolution of postzygotic isolation in the lab and to dissect its genetic basis. Here we describe several genetic and developmental analyses that led to timing the spread of this severe F₁ hybrid embryonic lethality within eight years of laboratory maintenance.

Methods

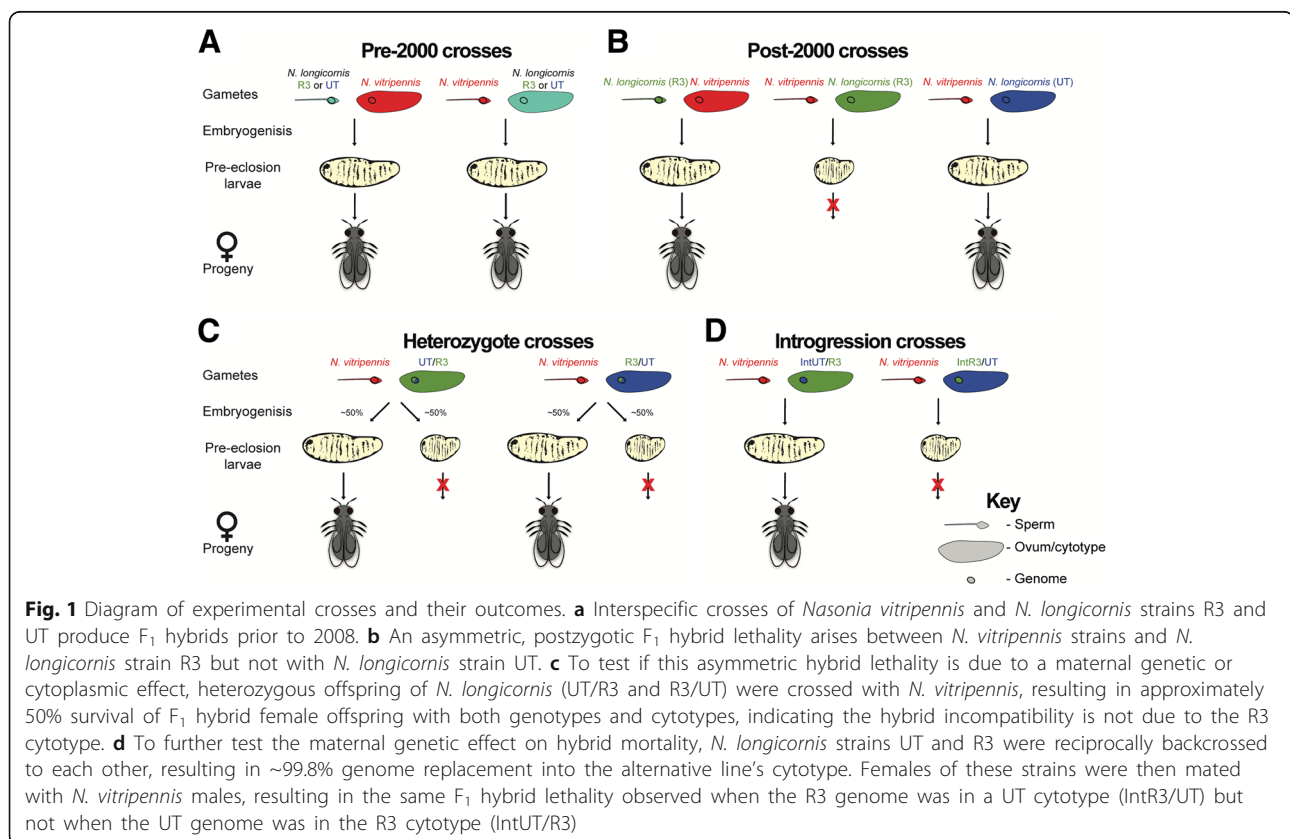
Strains

All *Nasonia* wasps were reared in 25 °C incubators with constant light on *Sarcophaga bullata* fly pupae ('hosts')

raised in the lab. All fly hosts were always checked for color and firmness prior to providing them to adult female *Nasonia* to ensure the quality was sufficient for parasitism. This included a dark amber to brown puparium with yellow, firm to the touch, fly pupae inside (approximately 13 days old post egg laying). Two strains of *N. longicornis* were used in this study: IV7R3-1b (R3) and NAS_NLUT230A (UT). Strain IV7R3-1b is derived from strain IV7, a *Wolbachia* infected strain that was collected in Utah and antibiotically cured of *Wolbachia* in 2000 [16]. NAS_NLUT230A was collected from a natural population in Utah between 1989 and 1991, cured of *Wolbachia* by antibiotic treatment, and maintained in the laboratory according to methods described previously [22]. Two strains of *N. vitripennis* were used in this study: 13.2 and 12.1T. Strain 13.2 was derived from the R511 line collected in New York and cured of *Wolbachia* through a period of prolonged diapause in 1996 [23]. The *Wolbachia*-uninfected 12.1T strain was derived after antibiotic treatment from strain 12.1 that harbors a *Wolbachia* infection [24] and originally derived from R511 [23]. The generation time for *N. longicornis* and *N. vitripennis* under these rearing conditions is approximately two weeks.

Collecting and mating *Nasonia*

All *Nasonia* stocks were matured into the yellow pupa stage at approximately twelve days of age and then



separated according to sex to ensure virginity. After pupae eclosion, adult wasps were allowed to feed on small amounts of honey. For experimental mating crosses, one adult male and one virgin adult female *Nasonia* of the desired strains were each placed into a glass test-tube vial that was capped with a cotton plug. The pairs were observed for copulation for 5–10 min. After copulation, females were provided with honey and one to two hosts, depending on the experiment for parasitizing and then incubated under constant light at 25 °C.

F₁ egg, larva, and pupa counts

Each mated female was provided honey and one unparasitized fly host with only the anterior end of the fly pupa protruding from a foam plug. This restricted the females' ovipositing and thus localized egg laying to the anterior region of the fly pupae. Twenty-four hours later, the fly hosts were removed for offspring counts and replaced in the foam plug with new, unparasitized hosts. To characterize the F₁ hybrid incompatibility, we reciprocally crossed *N. vitripennis* (strain 13.2) and *N. longicornis* (strain IV7R3-1b, hereafter referred to as R3) to produce F₁ hybrids (Fig. 1b). Concurrently, we set up control self-crosses. All mating pairs are denoted as male × female. For all crosses, females were hosted once with two hosts for 24 h, and then these hosts were placed in the incubator. The *Nasonia* developed into pupae over 12–18 days before pupa counts were performed, Fig. 2a (13.2 × 13.2, *n* = 8; R3 × 13.2, *n* = 3; 13.2 × R3, *n* = 5; R3 × R3, *n* = 5). For all counts, some offspring were not counted until they had already emerged from the pupal stage to adulthood. For the experimental crosses in Fig. 2b, the females were hosted twice, and one hosting was used for pupa counts (13.2 × 13.2, *n* = 23; 12.1T × 12.1T, *n* = 13; 13.2 × R3, *n* = 23; 12.1T × R3, *n* = 23; R3 × R3, *n* = 25).

To validate the strong F₁ hybrid reduction and examine its dependency on strain background, we set up two interspecific crosses using *N. longicornis* R3 females and either *N. vitripennis* 13.2 or 12.1T males that originated from the same inbred *N. vitripennis* strain in 1996 [23] (Fig. 2b). For the crosses in Fig. 2b after the hatch mark, the females were hosted six times with the fifth set used for pupa counts (Fig. 2b, 13.2 × 13.2, *n* = 5; 13.2 × UT, *n* = 5; UT × UT, *n* = 14).

The severe reduction of F₁ hybrids could be due to either a decrease in egg production or hybrid mortality during early developmental periods. The second set of hostings was used for egg counts to test for differences in fecundity (Fig. 2c, R3 × R3, *n* = 17; 13.2 × R3, *n* = 12; 13.2 × UT, *n* = 5; UT × UT, *n* = 15). Egg counts entail carefully puncturing the fly's puparium, removing its anterior portion, and then counting all *Nasonia* eggs observed on the fly body and in the removed section of the puparium. The third of these hostings was used for first instar larva counts to test the nature of the reduction, (Fig. 2c, R3 × R3, *n* = 18; 13.2 × R3, *n* = 10; 13.2 × UT, *n* = 6; UT × UT, *n* = 19). To calculate the number of surviving first instar larvae, the same procedure for egg counts was used except that the *Nasonia* embryos were allowed to develop undisturbed on the host for one and a half days before being counted.

Next, we determined if the laboratory-evolved asymmetric hybrid mortality is due to a cytonuclear interaction or maternal genetic effect by generating reciprocal F₁ heterozygous females between *N. longicornis* strains R3 and the compatible UT strain, resulting in identical nuclear genomes but cytotypes derived from either R3 or UT (Fig. 1c). For the heterozygous experimental crosses, one half of the hosts for each cross were used for egg counts the day after hosting and the other half were incubated for another 12 days and used for pupa counts. The same procedure was used for the

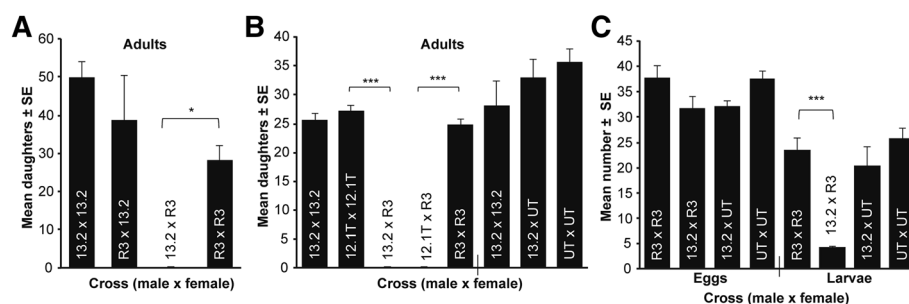


Fig. 2 F₁ hybrid mortality is asymmetric and embryonic. **a** Complete F₁ hybrid reduction occurs between *N. vitripennis* males (strain 13.2) and *N. longicornis* females (strain R3). Data are represented as mean number of surviving F₁ females (diploid) ± standard error (SE), Kolmogorov-Smirnov (K-S) test, **P* < 0.05. **b** The factor responsible for complete F₁ hybrid reduction in *N. vitripennis* is common to *N. vitripennis* strains 13.2 and 12.1T that shared an ancestor in 1996. Conversely, the factor responsible for complete F₁ hybrid reduction in *N. longicornis* is specific to the Utah-derived strain R3, but not UT. Data are F₁ females ± SE, K-S test, ****P* < 0.0001. **c** Complete F₁ hybrid reduction is due to hybrid mortality during embryonic development. Data are shown as mean number of F₁ eggs and 1st instar larvae ± SE, K-S test, ****P* < 0.0001

second hosting except the hosting group halves were switched. The hostings for pupae in Fig. 3a came after the hostings for pupae in Fig. 3b (Fig. 3a, UT × UT/R3, $n = 14$; 13.2 × UT/R3, $n = 14$; 13.2 × R3/UT, $n = 13$; R3 × R3/UT, $n = 11$; Fig. 3b UT × UT/R3, $n = 9$; 13.2 × UT/R3, $n = 12$; 13.2 × R3/UT, $n = 10$; R3 × R3/UT, $n = 9$).

To corroborate the interpretation of a maternal effect hybrid incompatibility, we generated two *N. longicornis* introgression lines by backcrossing the R3 nuclear genotype into the UT cytochrome (denoted IntR3/UT) and reciprocally the UT nuclear genotype into the R3 cytochrome (denoted IntUT/R3) for nine generations each (Fig. 1d). These introgression lines harbor ~99.8% of the nuclear genotype of one *N. longicornis* strain while maintaining the cytochrome of the alternate strain. If the hybrid mortality is due to a maternal effect in hybrid embryos, then only the introgression line with the R3 nuclear DNA will result in hybrid death. For the introgression crosses, females were hosted six times with the fifth set used for pupa counts (Fig. 5a, IntUT/R3 × IntUT/R3, $n = 16$; 13.2 × IntUT/R3, $n = 12$; 13.2 × IntR3/UT, $n = 7$; IntR3/UT × IntR3/UT, $n = 17$). Using introgression-heterozygotes we can retest the observations of Fig. 4 but with introgressed genomes within the alternative *N. longicornis* cytochromes. For the introgression-heterozygote crosses, we generated reciprocal F₁ heterozygotes by backcrossing the introgression lines to males of their cytochrome to generate R3/(IntUT/R3) and UT/(IntR3/UT) hybrids derived from the genotypes of father/mother (Fig. 5b).

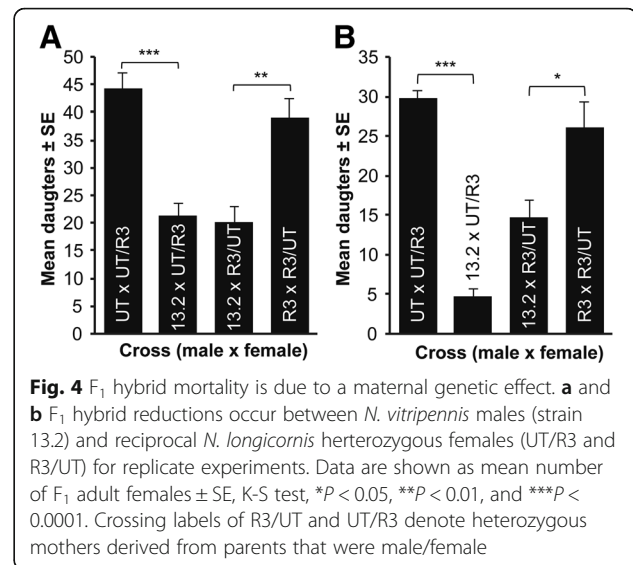


Fig. 4 F₁ hybrid mortality is due to a maternal genetic effect. **a** and **b** F₁ hybrid reductions occur between *N. vitripennis* males (strain 13.2) and reciprocal *N. longicornis* heterozygous females (UT/R3 and R3/UT) for replicate experiments. Data are shown as mean number of F₁ adult females ± SE, K-S test, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.0001$. Crossing labels of R3/UT and UT/R3 denote heterozygous mothers derived from parents that were male/female

Females were hosted five times with the second set of hosts used for pupa counts as previously described (Fig. 5b, R3/(IntUT/R3) × R3/(IntUT/R3), $n = 19$; 13.2 × R3/(IntUT/R3), $n = 16$; 13.2 × UT/(IntR3/UT), $n = 21$; UT/(IntR3/UT) × UT/(IntR3/UT), $n = 20$).

Statistical analysis

Crosses that produced no offspring were excluded from the counts, likely due to poor fly host quality (average of

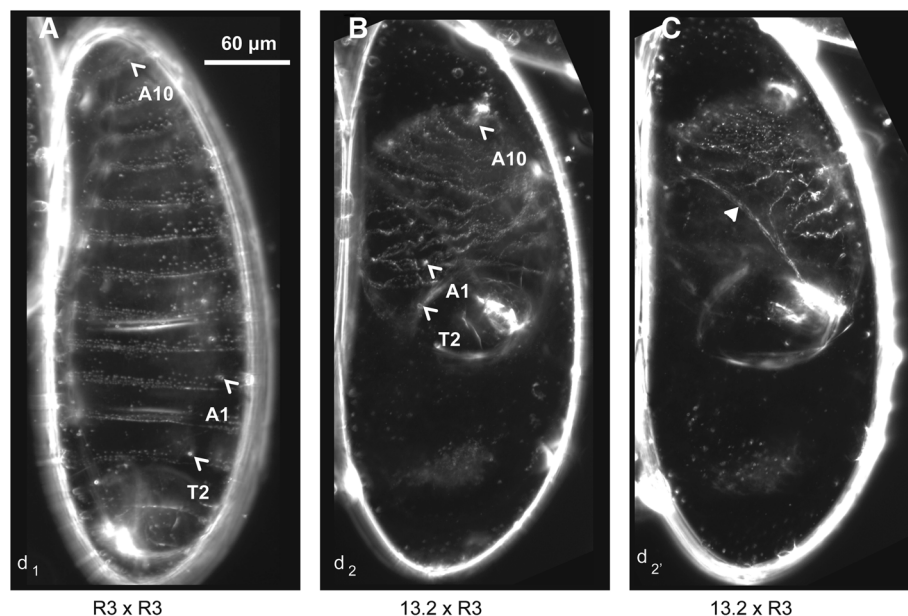
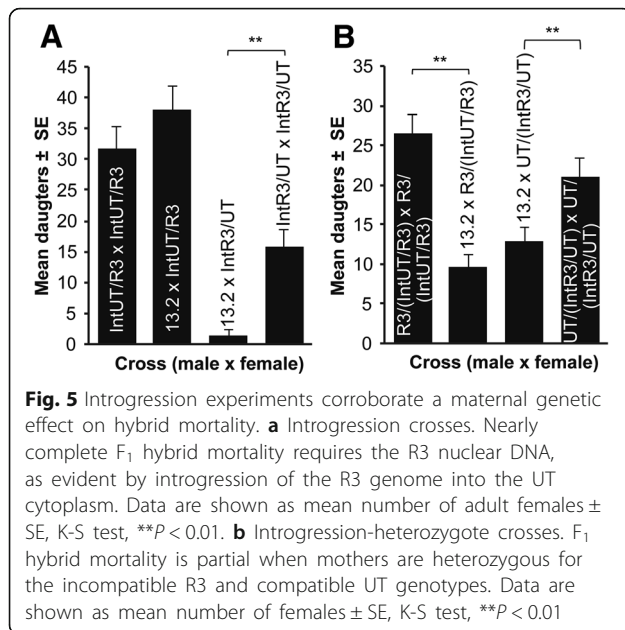


Fig. 3 Dark field imagery of 36 h embryos. **(a, d₁)** An R3 × R3 larval cuticle just before hatching (pre-eclosure), T₂ arrow indicates 2nd thoracic spiracle, A₁ arrow indicates 1st abdominal spiracle, A₁₀ arrow indicates 10th abdominal deticle belt. **(b, d₂)** **a** representative unhatched 13.2 × R3 cuticle at two focal planes. **(c, d₂)** An unhatched 13.2 × R3 cuticle at two focal planes, closed arrow in indicates the edge of cuticle bordering the dorsal hole indicating a failure in dorsal closure



4.9% \pm 2.1%). Crosses in which failure of fertilization occurred, as evident by all male offspring, were also excluded. Crosses that produced more than three offspring that were in diapause or were otherwise unidentifiable by sex were excluded from further analysis to avoid artifacts in the analysis because sex is unknown in the diapaused larvae. Kolmogorov-Smirnov (K-S) tests were used to compare statistical distributions of the number of daughters between each interspecific cross and corresponding intraspecific control cross for each experiment. The statistical package JMP 11 was used to perform Kolmogorov-Smirnov tests to compare egg production, female adult production, male adult production, and larva production between each interspecific cross and corresponding intraspecific control cross.

Testing for *Wolbachia* infection

To confirm the uninfected *Wolbachia* status of the *Nasonia* strains used, DNA was extracted using the Genra PureGene DNA extraction kit (QIAGEN[®]), as well as a control infected strain (*N. vitripennis* 12.1). Verification of infection status was performed using PCR with the primers ftsZuniF and fts2uniR as previously described [25]. Individual adult *Nasonia*, $n = 4$ UT, $n = 3$ R3, and $n = 3$ 13.2, $n = 3$ *Wolbachia* positive *Nasonia* - strain 12.1, were tested and no *Wolbachia* was detected in the UT, R3, or 13.2 strains.

Cuticle prep methods

To test embryonic and pre-eclosing larval development, eggs were collected from three R3 \times R3 and three 13.2 \times R3 mated females. Mated females laid eggs overnight at 25 °C in individual egg laying chambers. Eggs were

collected to 1% PBS/agarose plates and incubated at 25 °C for ~48 h. Unhatched eggs and empty cuticles were counted from each type of mating. Unhatched eggs were transferred to a drop of 90:10 lactic acid:ethanol on a glass slide, covered with 22x22mm cover glass and baked at 65 °C overnight. Some R3 \times R3 eggs were collected and prepared after ~30 h to catch them before hatching. Cleared cuticles were observed under dark field optics. Contrast and brightness were enhanced using Adobe Photoshop[®].

Results

All mating pairs are denoted as male \times female. Figure 2a shows that while there are no significant differences in hybrids produced in the interspecific cross R3 \times 13.2 and control cross 13.2 \times 13.2 (Kolmogorov-Smirnov, $P = 0.97$, hereafter referred to as K-S), there is a significant and marked lack of hybrids observed in the reciprocal interspecific cross 13.2 \times R3 compared to control cross R3 \times R3 (K-S, $P = 0.014$). Thus, F_1 hybrid reduction is contingent on having a 13.2 father and R3 mother. Due to the haplodiploid sex determination of *Nasonia*, F_1 haploid sons are not hybrids, develop from unfertilized eggs, and arise from the maternal genotype. Accordingly, in all of the inter- and intraspecific crosses above and hereafter, there are no significant differences in F_1 male survival (Fig. 2a, 13.2 \times R3 compared to R3 \times R3, K-S, $P = 0.769$; 13.2 \times R3 and 12.1T \times R3 compared to R3 \times R3, K-S, $P = 0.969$ and $P = 0.405$ respectively).

For the two interspecific crosses using *N. longicornis* R3 females and either *N. vitripennis* 13.2 or 12.1T (Fig. 2b) males, there were no hybrids produced. Conversely, the R3 \times R3 control cross yielded normal numbers of offspring (Fig. 2b, K-S, $P < 0.0001$ for both comparisons). Since the underlying genetic factor for the hybrid reduction is presumably in both the *N. vitripennis* 13.2 and 12.1T strains, it is likely that the incompatibility originated prior to their 1996 splitting [23]. Next, we assessed whether the incompatibility in *N. longicornis* is strain dependent by crossing 13.2 males with females from a second *N. longicornis* strain, NAS_NLUT 230A (hereafter referred to as UT). The UT and R3 strains were collected in Utah several decades ago. Figure 2b shows that there was no reduction in F_1 hybrids in the 13.2 \times UT cross compared to the control UT \times UT cross (K-S, $P = 0.6896$). Therefore, the F_1 hybrid reduction is specific to the extant strain R3 that was previously compatible with the same *N. vitripennis* strain 13.2 in the year 2000 [16]. Likewise, when R3 was reestablished from larval diapause stocks from 2008, we again observed F_1 hybrid reduction in the 13.2 \times R3 interspecific cross compared to that of the self R3 \times R3 (K-S, $P = 0.0135$). Thus, we calibrate the spread of the *N.*

longicornis R3 incompatibility factor to an eight-year time span between the years 2000 and 2008.

For egg counts, we observed no significant differences in fecundity between the interspecific cross 13.2 × R3 and the control cross R3 × R3 (Fig. 2c, K-S, $P = 0.22$). Thus, the decrease in F₁ hybrids is due to postzygotic hybrid mortality. We observed a significant 82.4% reduction in the first instar larvae in the 13.2 × R3 cross relative to the R3 × R3 control (Fig. 2c, K-S, $P < 0.0001$), indicating the hybrid mortality is primarily embryonic; the surviving larvae are haploid males. Embryonic mortality is also evident by microscopy in which 12/65 progeny from the R3 × R3 crosses failed to hatch after more than 36 h, while 66/70 from the 13.2 × R3 hybrid eggs failed to hatch (Chi-squared test, $P < 0.0001$). The hatched embryos were haploid males and not hybrids. Of the unhatched hybrid embryos, many were tiny, malformed, and clearly inviable. The cuticles of the inviable larvae had all three thoracic and ten abdominal segments, but were highly compressed (Fig. 3). Many also showed large openings on the dorsal side of the cuticle, indicating a failure in dorsal closure. Similar phenotypes have been observed in screens for embryonic lethal mutations in *Nasonia* [26] and *Drosophila* [27]. These results imply that hybrid lethality likely affects a specific developmental process, such as dorsal-ventral patterning or extraembryonic membrane specification.

The *N. longicornis* heterozygous genotypes are denoted UT/R3 and R3/UT and represent paternal/maternal origins. We crossed these heterozygous females with *N. vitripennis* 13.2 males in two replicate experiments, and observed significant hybrid reductions in crosses to both R3/UT or UT/R3 females (Fig. 4a and b). These results exclude the R3 cytotypic causing hybrid mortality, and are consistent with a maternal genetic effect incompatibility between the maternal R3 genotype and 13.2 embryonic genotype, irrespective of the *N. longicornis* cytotypic.

The *N. longicornis* introgression lines used to test a maternal effect on hybrid incompatibility, IntR3/UT and IntUT/R3, exhibited an asymmetric F₁ hybrid lethality when crossed to *N. vitripennis* 13.2 males (Fig. 5a). Specifically, 92% hybrid mortality occurred in the incompatible cross with *N. longicornis* females containing a majority R3 genome and a UT cytoplasm (IntR3/UT). In contrast, *N. longicornis* females with the UT genome and R3 cytoplasm produced slightly more offspring (hybrids) than the control self-cross. These and the aforementioned results validate the contingency of the hybrid mortality on a R3 nuclear genotype expressed maternally during oogenesis, that in turn negatively interacts with *N. vitripennis* nuclear genes in the embryo.

We found no hybrid lethality in the crosses with heterozygous or introgression lines between the two *N.*

longicornis strains, indicating that the incompatibility arose specifically between R3 and *N. vitripennis*. It is important to note that although the control IntR3/UT cross in Fig. 5a is not as fecund as the IntUT/R3 line, giving the appearance of a potential cytonuclear incompatibility between R3 genotype and UT cytotypic, there is no significant difference between egg and adult production for this line (K-S, $P = 0.675$). Therefore, the incompatibility exhibited between species is not observed between strains within the *N. longicornis* species.

In Fig. 5b, we generated reciprocal F₁ heterozygotes using the introgression lines, R3/(IntUT/R3) and UT/(IntR3/UT); these heterozygous genotypes contain different *N. longicornis* cytotypes, but the same *N. longicornis* heterozygous nuclear genotypes. If hybrid mortality with 13.2 males is due to the proposed interaction between the R3 maternal genotype and *N. vitripennis* 13.2 embryonic genotype, then *N. longicornis* heterozygous mothers between R3 and UT should again yield partial mortality. Indeed, Fig. 5b shows that there was approximately a 50% reduction of daughters produced in both cross directions, as expected.

Discussion

The experiments presented here demonstrate, for the first time, that complete F₁ hybrid lethality evolved in the laboratory between *N. vitripennis* males and *N. longicornis* females between 2000 [20] to 2008. Assuming a two-week generation time, the incompatibility spread in less than 209 generations in cultures typically maintained with less than thirty foundresses per generation. The F₁ hybrid mortality is in part due to a *N. longicornis* R3 maternal genetic effect, rather than the R3 cytotypic. This conclusion is principally based on experiments crossing *N. vitripennis* males to reciprocal F₁ heterozygous females between *N. longicornis* strains R3 and the compatible UT strain that vary in cytotypic. If hybrid mortality was due to a cytonuclear interaction - which is common in F₂ hybrid males of *Nasonia* [18–20] - then hybrid mortality would have been unidirectional and contingent on parental females harboring the R3 cytotypic. Instead, we observed hybrid mortality in both crosses. Moreover, there mortality appears to involve an interaction between an embryonic product of the *N. vitripennis* genome and a maternal product of the R3 genome that is partially dominant or codominant with the maternal UT allele(s), since approximately half of the progeny of the heterozygous *N. longicornis* (UT/R3 or R3/UT) females succumb to lethality. Given that the embryo lethality seems to be related to a fairly narrow set of developmental processes (the tightly intertwined dorsoventral (DV) patterning and extraembryonic membrane specification processes) [28], a single locus developmental gene (e.g., transcription factor or signal

transduction pathway component) would be a plausible genetic part of the hybrid incompatibility, though this inference awaits future experimentation.

It is possible that a polymorphism within the species of *N. longicornis* ultimately spread in the R3 line via correlated selection or drift to cause the F₁ hybrid incompatibility, as reviewed in [29]. While selection or drift may have fixed the polymorphism from standing genetic variation in the species, the relatively few foundresses that established the R3 line and the subsequent inbreeding and bottlenecking of laboratory stocks likely reduced genetic variability and had an influence on the relatively sudden fixation within the laboratory. Previous laboratory experiments on the evolution of reproductive isolation found that selection can drive fixation of traits and reproductive barriers in a short period of time (as reviewed in [30, 31]). Experimental evolution of *Drosophila melanogaster* on a diet of EDTA led to a high degree of variability in fitness/fecundity in F₁ hybrid crosses between the control and adapted strains [32]. Following these observations, several generations of introgression switched the third chromosome of a control strain into the background of the EDTA-adapted strain, resulting in complete sterility of females on all diets as well as lethality of both sexes at high-EDTA conditions.

While the sudden appearance of genetically-based hybrid embryonic lethality in animal hybrids is very rare, embryonic lethality in hybrid embryos occurs in other insects. For instance, several interspecific crosses within the *Drosophila* genus lead to embryonic lethality [33–35]. Similar to the incompatibility in *Nasonia*, these defects are often sex specific and depend on the direction of the cross. For example, *D. melanogaster* males × *D. simulans* females produce lethality in female embryos [34], *D. melanogaster* females × *D. santomea* males produce dead male embryos [35], and *D. montana* females × *D. texana* males leads to complete female embryo lethality in early development [33]. Unlike *Nasonia*, which lack sex chromosomes, the sex specificity in *Drosophila* seems to arise from negative interactions between the X-chromosome of one species and autosomal loci of the other. In addition, the *Drosophila* species pairs diverged 3–11 million years ago while *N. longicornis* and *N. vitripennis* diverged approximately one million years ago.

Despite these major differences between insect systems, some observations in *Drosophila* are relevant for the rapid appearance of hybrid embryonic lethality observed in *Nasonia*. A survey of *D. simulans* and *D. santomea* populations found that there is natural variation affecting the strength or presence of the hybrid embryonic lethality when crossed to *D. melanogaster* [35, 36]. These and other cases in *Arabidopsis* [11] and *Tribolium* [12, 37], suggest that alleles affecting hybrid incompatibilities are segregating within

populations. However, it is unclear if within-species genetic variation for hybrid incompatibilities spread recently or in the distant past. The rate at which lineages evolve intrinsic postzygotic isolation has been benchmarked against the rate of prezygotic isolation evolution [38–41]. For example, strains of allopatric *Drosophila* can have equal rates of divergence for pre- and post-zygotic reproductive isolation, while birds develop postzygotic barriers much slower [38, 41, 42]. Extremely small population sizes and relatively short time frames have also never been linked to the evolution of hybrid mortality [31]. This lack of knowledge on the tempo of evolutionary genetic changes affecting hybrid incompatibilities suggests that the spread of alleles underlying genetically-based hybrid mortality may be unusually prolonged in time and/or restricted to large populations.

In contrast, there is some evidence for the rapid evolution of bottleneck-induced premating isolation [1, 4, 43]. Various speciation theories propose that evolution (i.e., selection and/or genetic drift) in small demes can cause the spread of a particular set of traits and genetic make-up, which may lead to strong reproductive isolation [44]. For instance, hybrid lethality between species could suddenly arise if selection or population bottlenecks fix segregating variation for hybrid lethality. In the present work, we observed the rapid evolution of a complete, asymmetric, F₁ hybrid mortality within eight years of routine laboratory rearing. These laboratory populations are maintained as small, inbred populations where strong selection or genetic drift may have rapidly promoted the spread of allele(s) for complete F₁ inter-specific hybrid lethality.

Conclusions

Here we have characterized the establishment of severe F1 hybrid mortality in observable time. The asymmetric incompatibility spread in less than eight years under laboratory maintenance of small populations of a strain of *N. longicornis* and is likely due to a negative interaction between a strain-specific maternal genetic effect and a nuclear-encoded product derived from *N. vitripennis* in F1 hybrid embryos. Hybrid mortality appears related to a disruption of early embryonic patterning, where maternal effects and the zygotic genome first interact.

Abbreviations

Int: Introgression; K-S: Kolmogorov-Smirnov; R3: IV7R3-1b; UT: NAS_NLUT230A

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Availability of data and materials

All stock lines are maintained at Vanderbilt University and University of Chicago and available upon request.

Authors' contributions

AS contributed to the design and execution of the experimental matings and embryogenesis observations as well as writing the manuscript. MP contributed to the execution of experimental matings. AN contributed by conducting the diapause line experimental matings. JAL contributed by conducting the embryogenesis work and writing the manuscript, SRB contributed to the design of the experiments and the writing of the manuscript. RMB contributed to the design and execution of the experimental mating crosses and embryogenesis observations as well as writing the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval

Not applicable.

Author details

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References

- Crossley SA. Changes in mating-behavior produced by selection for ethological isolation between ebony and vestigial mutants of *Drosophila melanogaster*. *Evolution*. 1974;28(4):631–47.
- del Solar E. Sexual isolation caused by selection for positive and negative phototaxis and geotaxis in *Drosophila pseudoobscura*. *Proc Natl Acad Sci U S A*. 1966;56(2):484–7.
- Kilias G, Alahiotis SN, Pelecanos M. A multifactorial genetic investigation of speciation theory using *Drosophila melanogaster*. *Evolution*. 1980;34(4):730–7.
- Dodd DMB. Reproductive isolation as a consequence of adaptive divergence in *Drosophila pseudoobscura*. *Evolution*. 1989;43(6):1308–11.
- Dettman JR, Sirjusingh C, Kohn LM, Anderson JB. Incipient speciation by divergent adaptation and antagonistic epistasis in yeast. *Nature*. 2007;447(7144):585–8.
- Curnoe D, Thorne A, Coate JA. Timing and tempo of primate speciation. *J Evolution Biol*. 2006;19(1):59–65.
- Fitzpatrick BM. Rates of evolution of hybrid inviability in birds and mammals. *Evolution*. 2004;58(8):1865–70.
- Price TD, Bouvier MM. The evolution of F1 postzygotic incompatibilities in birds. *Evolution*. 2002;56(10):2083–9.
- Sasa MM, Chippindale PT, Johnson NA. Patterns of postzygotic isolation in frogs. *Evolution*. 1998;52(6):1811–20.
- Lijtmaer DA, Mahler B, Tubaro PL. Hybridization and postzygotic isolation patterns in pigeons and doves. *Evolution*. 2003;57(6):1411–8.
- Bombliès K, Weigel D. Hybrid necrosis: autoimmunity as a potential gene-flow barrier in plant species. *Nat Rev Genet*. 2007;8(5):382–93.
- Wade MJ, Johnson NA. Reproductive isolation between two species of flour beetles, *Tribolium castaneum* and *T. freemani*: variation within and among geographical populations of *T. castaneum*. *Heredity*. 1994;72:155–62.
- Raychoudhury R, Desjardins CA, Buellesbach J, Loehlin DW, Grillenberger BK, Beukeboom L, Schmitt T, Werren JH. Behavioral and genetic characteristics of a new species of *Nasonia*. *Heredity*. 2010;104(3):278–88.
- Dittmer J, van Opstal EJ, Shropshire JD, Bordenstein SR, Hurst GDD, Brucker RM. Disentangling a Holobiont - recent advances and perspectives in *Nasonia* wasps. *Front Microbiol*. 2016;7:1478.
- Bordenstein SR, O'Hara FP, Werren JH. Wolbachia-induced incompatibility precedes other hybrid incompatibilities in *Nasonia*. *Nature*. 2001;409(6821):707–10.
- Bordenstein SR, Werren JH. Bidirectional incompatibility among divergent *Wolbachia* and incompatibility level differences among closely related *Wolbachia* in *Nasonia*. *Heredity*. 2007;99(3):278–87.
- Beukeboom LW, van den Assem J. Courtship and mating behaviour of interspecific *Nasonia* hybrids (Hymenoptera, Pteromalidae): A grandfather effect. *Behav Genet*. 2001;31(2):167–77.
- Breeuwer JAJ, Werren JH. Hybrid breakdown between two haplodiploid species: the role of nuclear and cytoplasmic genes. *Evolution*. 1995;49(4):705–17.
- Koevoets T, Niehuis O, van de Zande L, Beukeboom LW. Hybrid incompatibilities in the parasitic wasp genus *Nasonia*: negative effects of hemizygoty and the identification of transmission ratio distortion loci. *Heredity*. 2012;108(3):302–11.
- Niehuis O, Judson AK, Gadau J. Cytonuclear genic incompatibilities cause increased mortality in male F2 hybrids of *Nasonia giraulti* and *N. vitripennis*. *Genetics*. 2008;178(1):413–26.
- Bordenstein SR, Drapeau MD, Werren JH. Intraspecific variation in sexual isolation in the jewel wasp *Nasonia*. *Evolution*. 2000;54(2):567–73.
- Brucker RM, Bordenstein SR. The hologenomic basis of speciation: gut bacteria cause hybrid lethality in the genus *Nasonia*. *Science*. 341(6146):667–69.
- Perrot-Minnot MJ, Guo LR, Werren JH. Single and double infections with *Wolbachia* in the parasitic wasp *Nasonia vitripennis*: Effects on compatibility. *Genetics*. 1996;143(2):961–72.
- Chafee ME, Zecher CN, Gourley ML, Schmidt VT, Chen JH, Bordenstein SR, Clark ME, Bordenstein SR. Decoupling of host-symbiont-phage coadaptations following transfer between insect species. *Genetics*. 2011;187(1):203–15.
- Baldo L, Dunning Hotopp JC, Jolley KA, Bordenstein SR, Biber SA, Choudhury RR, Hayashi C, Maiden MC, Tettelin H, Werren JH. Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. *Appl Environ Microbiol*. 2006;72(11):7098–110.
- Pultz MA, Zimmerman KK, Alto NM, Kaerberlein M, Lange SK, Pitt JN, Reeves NL, Zehrung DL. A genetic screen for zygotic embryonic lethal mutations affecting cuticular morphology in the wasp *Nasonia vitripennis*. *Genetics*. 2000;154(3):1213–29.
- Jurgens G, Wieschaus E, Nussleinvolhard C, Kluding H. Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. II. Zygotic loci on the third chromosome. *Roux Arch Dev Biol*. 1984;193(5):283–95.
- Ozuak O, Buchta T, Roth S, Lynch JA. Dorsoventral polarity of the *Nasonia* embryo primarily relies on a BMP gradient formed without input from toll. *Curr Biol*. 2014;24(20):2393–8.
- Cutter AD. The polymorphic prelude to Bateson-Dobzhansky-Muller incompatibilities. *Trends Ecol Evol*. 2012;27(4):209–18.
- Rice WR, Hostert EE. Laboratory experiments on speciation: what have we learned in 40 years? *Evolution*. 1993;47(6):1637–53.
- Odeen A, Florin AB. Effective population size may limit the power of laboratory experiments to demonstrate sympatric and parapatric speciation. *Proc Biol Sci*. 2000;267(1443):601–6.
- Robertson FW. The ecological genetics of growth in *Drosophila*. 8. Adaptation to a new diet. *Genet Res*. 1966;8(2):165–79.
- Kinsey JD. Studies on an embryonic lethal hybrid in *Drosophila*. *Development*. 1967;17(2):405–23.
- Hadom E. Zur Autonomie und Phasenspezifität der Letalität von Bastarden zwischen *Drosophila melanogaster* und *Drosophila simulans*. Genève: Kundig; 1961.
- Gavin-Smyth J, Matute DR. Embryonic lethality leads to hybrid male inviability in hybrids between *Drosophila melanogaster* and *D. santomea*. *Ecol Evol*. 2013;3(6):1580–9.
- Gerard PR, Presgraves DC. Abundant genetic variability in *Drosophila simulans* for hybrid female lethality in interspecific crosses to *Drosophila melanogaster*. *Genet Res*. 2012;94(1):1–7.
- Wade MJ, Johnson NA, Jones R, Siguel V, McNaughton M. Genetic variation segregating in natural populations of *Tribolium castaneum* affecting traits observed in hybrids with *T. freemani*. *Genetics*. 1997;147(3):1235–47.
- Coyne JA, Orr HA. "Patterns of speciation in *Drosophila*" Revisited. *Evolution*. 1997;51(1):295–303.
- Kozak GM, Rudolph AB, Colon BL, Fuller RC. Postzygotic isolation evolves before prezygotic isolation between fresh and saltwater populations of the rainwater killifish, *Lucania parva*. *Int J Evol Biol*. 2012;2012:523967.
- Yukilevich R. Asymmetrical patterns of speciation uniquely support reinforcement in *Drosophila*. *Evolution*. 2012;66(5):1430–46.
- Rabosky DL, Matute DR. Macroevolutionary speciation rates are decoupled from the evolution of intrinsic reproductive isolation in *Drosophila* and birds. *Proc Natl Acad Sci U S A*. 2013;110(38):15354–9.

42. Orr HA, Coyne JA. The genetics of postzygotic isolation in the *Drosophila virilis* group. *Genetics*. 1989;121(3):527–37.
43. Matute DR. The role of founder effects on the evolution of reproductive isolation. *J Evolution Biol*. 2013;26(11):2299–311.
44. Templeton AR. The theory of speciation via the founder principle. *Genetics*. 1980;94(4):1011–38.

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