



Genetic mechanisms preventing the fusion of ecotypes even in the face of gene flow

Issei Ohshima^{1,2,3*}

¹Department of Ecology and Systematics, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan, ²Division of Evolutionary Biology, National Institute for Basic Biology, Okazaki 444-8585, Japan, ³Department of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto 606-8522, Japan.

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Correspondence and requests for materials should be addressed to I.O. (issei@kpu.ac.jp)

* Current address:
Department of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto 606-8522, Japan.

Understanding the genetics behind adaptation and reproductive isolation contributes to our knowledge about how biodiversity is created and maintained. Host races of phytophagous insects are host-associated ecotypes and have been considered as candidates for ecological speciation, but very little is known about the genetic backgrounds of host adaptations. A leaf-mining moth, *Acrocercops transecta*, consists of *Juglans*- and *Lyonia*-associated host races. This study assesses the genetic bases of oviposition preference and larval performance using F₁, F₂ and backcross hybrids between the two host races. Segregation patterns in the hybrid generations revealed that larval performance on *Juglans* is dominant, but oviposition preference for *Lyonia* is dominant. This result indicates that genetic components introgressed from the *Lyonia* race are removed from the *Juglans* race even though hybrid larvae are viable on *Juglans*. Thus, simple genetic controls with contrasting dominance directions in host-adaptation traits function as barriers to prevent a fusion of host races.

Adaptation to a novel environment often requires the evolution of multiple traits, and hybridization between ecologically divergent taxa could produce maladaptive phenotype combinations, resulting in isolation barriers^{1–6}. In phytophagous insects, a precise combination of preference (e.g., ovipositing female preference) and performance (e.g., larval tolerance to secondary compounds) for particular host plants is crucial because a new host plant can be incorporated into an insect's diet only if adults accept it for oviposition and if the larvae are able to complete their development on it⁷. In most cases, preferences and performances in phytophagous insects are under genetic control⁸. Thus, differences in the mode of inheritance between these two traits may result in isolating barriers between host races.

To address the genetic mechanisms preventing the fusion of host races, I studied a leaf-mining moth, *Acrocercops transecta* (Gracillariidae), which consists of *Juglans* (Juglandaceae)- and *Lyonia* (Ericaceae)-associated host races (see Supplementary Background Text for details). The two host races clearly differ in the host preferences of ovipositing females and larval performances on host plants but mate readily in the laboratory, producing fertile hybrids⁹. Because the resistance to *Lyonia* is completely recessive to resistance to *Juglans*, the F₁ hybrid larvae can survive only when they feed on *Juglans*⁹, indicating that gene flow should be directed from the *Lyonia* race to the *Juglans* race. However, because F₁ larvae exhibit high viability on *Juglans*⁹, gene flow from the *Juglans* race to the *Lyonia* race is also possible if enclosed F₁ hybrid males mate with females of the *Lyonia* race. The *Juglans* and *Lyonia* races are often sympatric in the wild, and there is no phenological or host-associated premating isolation between them^{10,11}. Indeed, gene flow has occurred in both directions between the two host races in the wild^{12,13}. Thus, there should be postmating genetic mechanisms that maintain the differences between the two host races even in the face of gene flow.

Maladaptive alleles introgressed through hybridization are likely to be eliminated from respective host races, but the strength and extent of purifying selection against the alleles depend on the mode of inheritance of each locus. Although a previous study has revealed the direction of dominance of larval performance⁹, the segregation patterns in F₂- and backcross-hybrid larvae are still unknown. Further, the oviposition preferences in F₁-, F₂- and backcross-hybrid females are also still unknown. Thus, detailed observations of phenotypes in both oviposition preference and larval performance in hybrid generations are crucial to infer the genetic bases of host adaptation and to evaluate their contribution to an isolating barrier between the two host races of *A. transecta*.



To investigate the genetic bases of oviposition preference and larval performance, two sympatric and five allopatric host-associated populations (*Juglans* race: Sendai, Sapporo and Yamagata; *Lyonia* race: Sendai, Okazaki, Kyoto and Kirishima; see Supplementary Fig. S1 online) in Japan were used for the experiments. First, F₁, F₂ and all combinations of backcrosses were established using the sympatric populations (Sendai) (see Supplementary Fig. S2A–H online). Next, I further assessed the segregation patterns of oviposition preference using backcrosses between *Juglans* females and F₁ hybrid males (J♀ x JL♂ backcross) that were established from Sapporo (*Juglans* race) and Kyoto (*Lyonia* race) populations (see Supplementary Fig. S2I online). Finally, segregation patterns of larval performance were further assessed using backcrosses between F₁ hybrid females and *Lyonia* males and vice versa (JL♀ x L♂ and L♀ x JL♂ backcrosses, respectively). I used Yamagata (*Juglans* race) and Okazaki and Kirishima (*Lyonia* race) populations for establishing the backcrosses (see Supplementary Fig. S2J, K online).

The goal of this study is to reveal the modes of inheritance of adaptive traits and to uncover the genetic mechanisms causing isolating barriers between ecologically divergent taxa.

Results

Oviposition preferences in F₁ hybrid females. Reciprocal hybrids (JL and LJ) were assessed in this experiment. F₁ hybrid females significantly preferred to oviposit on *Lyonia* rather than *Juglans* regardless of the direction of crosses, except for two females from JL crosses that laid more eggs on *Juglans* than on *Lyonia* (JL $t = 7.471$, $df = 38$, $P = 5.727e-9$; LJ $t = 4.225$, $df = 4$, $P = 1.343e-2$; paired t -test) (Fig. 1A–D). Because all F₁ hybrids fed on *Juglans* during their larval stages, the results indicate that the oviposition preference of adult females is determined not by larval experiences but by genetic factors. The results also indicate that the loci determining oviposition preference are located on autosomes and that the preference for *Lyonia* is dominant over that for *Juglans*. Thus, I used only JL F₁ hybrids for establishing F₂ and backcross hybrids in the subsequent experiments.

Oviposition preferences in F₂ and backcross hybrid females.

Assuming that oviposition preference is determined by a single-locus, two-allele system and that the *Lyonia*-preferring allele is dominant to the *Juglans*-preferring allele, the expected segregation ratios are 1:3 (prefer to oviposit on *Juglans*:*Lyonia*) in F₂, 0:1 in JL x L and L x JL, and 1:1 in J x JL (JL x J backcross is lethal, see below). In each of the pooled JL x L, L x JL, and J x JL backcrosses in the Sendai population, hybrid individuals segregated in the expected ratio (Fig. 1G–N; Table 1A). All females preferred to oviposit on *Lyonia* in the JL x L (Sendai) and L x JL (Sendai) backcrosses (JL x L $t = 9.798$, $P = 1.202e-7$, $df = 14$; L x JL $t = 4.757$, $P = 1.828e-4$, $df = 17$; paired t -test) (Fig. 1G, H, M, N). In the J x JL (Sendai) backcross, 95 females preferred to oviposit on *Juglans* and 87 preferred *Lyonia*, although three females laid eggs evenly on *Juglans* and *Lyonia* (Fig. 1J). For the 95 females that preferred *Juglans*, the mean number of eggs deposited on *Juglans* was significantly larger than that on *Lyonia* (paired t -test, $t = 13.1666$, $P = 2.2e-16$, $df = 94$), and the 87 females that preferred *Lyonia* deposited significantly more eggs on *Lyonia* (paired t -test, $t = 10.9945$, $P = 2.2e-16$, $df = 86$) (Fig. 1K, L). These results are consistent with the hypothesis of Mendelian inheritance, with the dominance of a *Lyonia*-preferring allele in the preference gene.

However, F₂ (Sendai) and the J x JL backcross (Sapporo x Kyoto) demonstrated biased segregation ratios (Fig. 1F, P; Table 1A). One possible hypothesis to explain the deviation from the expected ratio in F₂ hybrids is that the preference gene is physically linked to performance genes. Because all F₂ hybrid larvae were reared on *Lyonia* because of the oviposition preference of F₁ hybrid females and because the resistance to *Lyonia* is completely recessive to resistance to *Juglans*⁹, approximately three-quarters of F₂ hybrid larvae failed to survive on *Lyonia* (Fig. 2; Table 1B). Thus, given the linkage between preference and performance loci, all F₂ hybrids possessing a *Juglans*-preferring allele died on *Lyonia*. In contrast, the biased segregation pattern in the J x JL backcross (Sapporo x Kyoto) is difficult to explain. One of the possible reasons for this deviation is the experimental condition: a shortage of fresh *Lyonia* leaves may have inhibited the oviposition of females because the experiments were

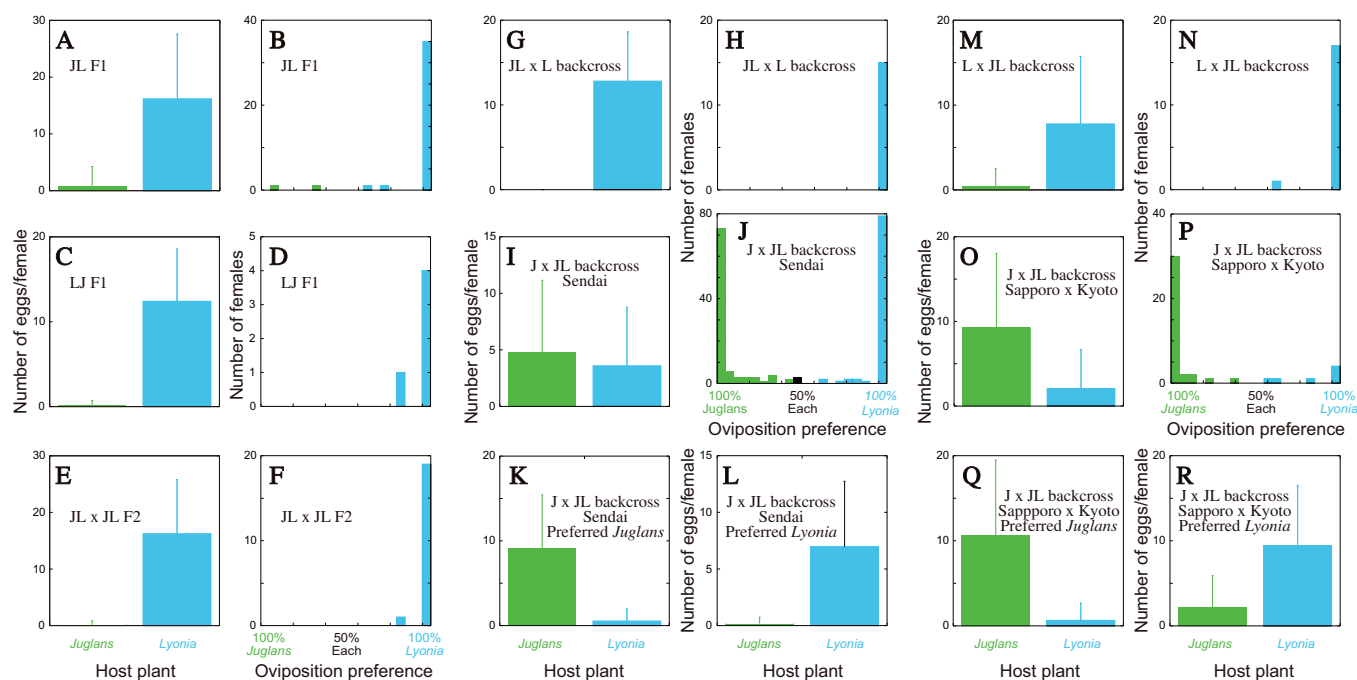


Figure 1 | Oviposition preference in F₁, F₂ and backcross hybrids. Mean values and standard deviations for the numbers of eggs laid (A, C, E, G, I, K, L, M, O, Q, R), and the individual ovipositing female preference (B, D, F, H, J, N, P) in each of the F₁, F₂ and backcross hybrids. In J x JL backcrosses, *Juglans*- or *Lyonia*-type females are separately illustrated because there were two types of females with regard to oviposition preference (K, L, Q, R).



Table 1 | Segregation patterns and single-locus goodness-of-fit tests in F_1 , F_2 and backcross hybrids. (A) oviposition preference. (B) larval performance.

(A)									
Population	Cross ^a	Total ^a	<i>Juglans</i> type ^b	<i>Lyonia</i> type ^b	Expected ^c	G	df	P-value	
Sendai	JL x JL F2	20	0	20	1:3	6.667	1	9.823e-3**	
	JL x L BC	15	0	15	0:1	0	1	1	
	J x JL BC	182	95	87	1:1	0.3516	1	0.5532	
	L x JL BC	18	0	18	0:1	0	1	1	
Sapporo x Kyoto	J x JL BC	43	36	7	1:1	19.56	1	9.758e-6***	
(B)									
Population	Cross ^a	Larval food ^d	Total ^a	Alive ^e	Dead ^e	Expected ^c	G	df	P-value
Sendai	JL x JL F2	<i>Lyonia</i>	343	76	267	1:3	1.4781	1	0.2241
	JL x J BC	<i>Lyonia</i>	157	0	157	0:1	0	1	1
	JL x L BC	<i>Lyonia</i>	240	113	127	1:1	0.8167	1	0.3662
	J x JL BC	<i>Juglans</i>	148	137	11	1:0	Infinite	1	< 2.2e-16***
	L x JL BC	<i>Lyonia</i>	328	155	173	1:1	0.9878	1	0.3203
Yamagata x Okazaki x Kirishima	JL x L BC	<i>Lyonia</i>	269	132	137	1:1	0.0929	1	0.7605
	L x JL BC	<i>Lyonia</i>	399	182	217	1:1	3.0702	1	0.07974

^aAll broods in each cross were pooled. No significant heterogeneity was observed within a given category of mating (see Materials and Methods).
^bFemale moths were considered to be a *Juglans* (or *Lyonia*) type if they laid more than half of their eggs on *Juglans* (or *Lyonia*).
^cExpected indicates the hypothesized segregation ratio for single-gene inheritance in each cross.
^dThe host plant on which larvae were maintained.
^eNumbers of hatched larvae that survived or did not survive until the second stadium.

conducted during winter and I used *Lyonia* plants maintained in a greenhouse. Alternatively, oviposition preference may be governed by several genes or influenced by maternal effects (e.g., symbionts) in the Sapporo population. There were much fewer females assessed in the J x JL backcross (Sapporo x Kyoto) than in the J x JL backcross (Sendai), so additional experiments with a larger sample size of J x JL backcross (Sapporo x Kyoto) females could reveal the factors for this deviation. However, what can be concluded is that *Lyonia*-preference is dominant to *Juglans*-preference and that a few loci control oviposition preference.

Larval performances in F_2 and backcross hybrid. Performance on *Juglans* is dominant over that on *Lyonia*⁹. Thus, assuming that larval performance is determined by a single-locus, two-allele system, the expected segregation ratios are 1:3 (survive:die on *Lyonia*) in F_2 , 0:1 (survive:die on *Lyonia*) in JL x J, 1:1 (survive:die on *Lyonia*) in JL x L and L x JL, and 1:0 (survive:die on *Juglans*) in J x JL backcrosses. All segregation patterns in F_2 and backcross hybrids supported the hypothesis except the J x JL backcross hybrids (Table 1B). However, the viability of the J x JL backcross was not significantly different from that of the control crosses (Fig. 2, see Supplementary

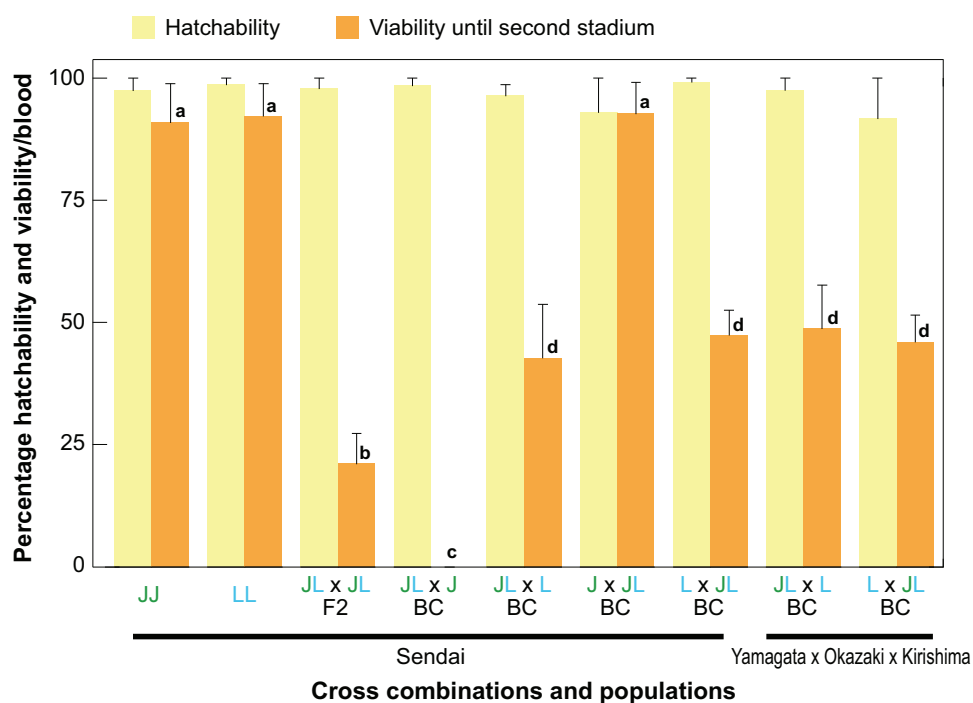


Figure 2 | Hatchability and viability until the second stadium in F_1 , F_2 , backcross hybrids, and pure *Juglans* or *Lyonia* races. For viability, a significant difference was found among crosses ($F_{8, 41} = 82.09$, $P < 2.2e-16$, one-way ANOVA). Thus, post hoc Tukey-Kramer HSD pairwise comparisons were performed between crosses. Different letters indicate significant differences ($P < 0.05$, Tukey-Kramer HSD test).



Table S1 online for detailed statistical results), suggesting that the slightly reduced viability was not due to the lack of the resistance to *Juglans* but due to accidental mortality. Thus, the present results indicate that larval performance is governed by a single-locus, two-allele system with complete dominance of resistance to *Juglans*. In addition, the hatchability of the eggs from every cross combination was higher than 90%. There were no significant differences in hatchability between pure races and hybrids ($F_{8, 41} = 0.7583$, $P = 0.6406$) (Fig. 2), indicating that there was not intrinsic reproductive isolation between the two host races.

Discussion

The present results provide evidence that the difference in both ovipositing female preference and larval performance between host races of *A. transecta* is each mainly determined by a single-locus, two-allele system with dominance, respectively. However, a few females showed an intermediate oviposition preference and a biased

segregation ratio in the J x JL backcross (Sapporo x Kyoto), implying the existence of modifier genes or maternal effects in host preference. The directions of dominance for female preference and larval performance were opposite, indicating that preference and performance are under different genetic controls.

The present findings have implications for the mechanism that prevent the fusion of the two host races even when gene flow occurs. The allele for resistance to *Juglans* is dominant. Thus, F₁ larvae could survive if *Juglans* females mate with *Lyonia* males, resulting in asymmetrical gene flow from the *Lyonia* race to the *Juglans* race (Fig. 3). However, eclosed F₁ females avoid ovipositing on *Juglans* because of the expression of the *Lyonia*-preferring allele. This result indicates that genetic components that introgressed from the *Lyonia* race were removed from the *Juglans* race (Fig. 3). Thus, the differences in the direction of dominance between preference and performance loci themselves function as a barrier to prevent the fusion of the two host races. A physical linkage between the preference and performance

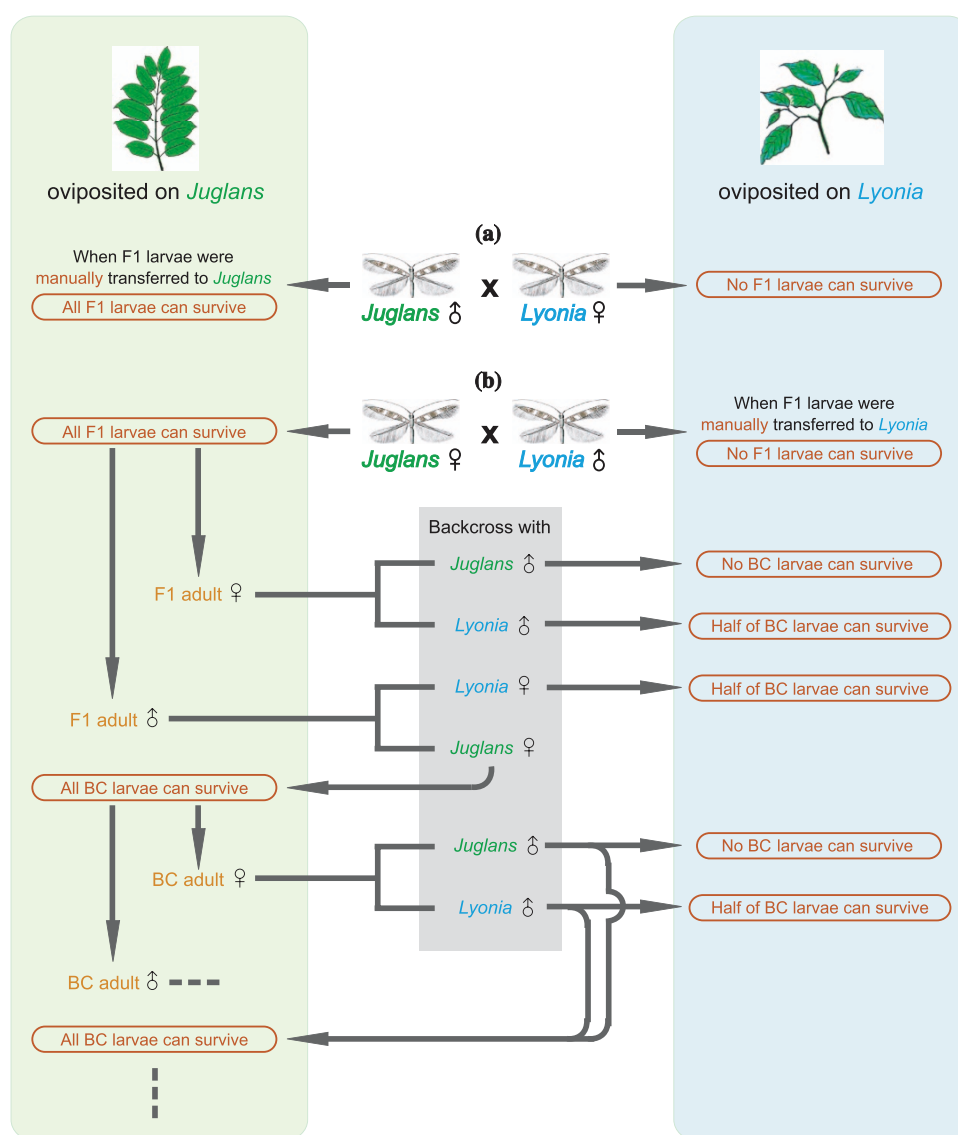


Figure 3 | Summary of the genetic mechanisms that prevent fusion of the two host races in *A. transecta*. (a) F₁ hybrid eggs from crosses between *Lyonia* females and *Juglans* males are deposited on *Lyonia*. However, F₁ hybrid larvae cannot survive on *Lyonia* because of an expression of the dominant *Juglans*-resistance allele. Thus, alleles for *Juglans* resistance and *Juglans* preference are eliminated from the *Lyonia* race immediately. (b) F₁ hybrid larvae from crosses between *Juglans* females and *Lyonia* males can develop to adulthood on *Juglans*, but no eclosed adult females prefer to oviposit on *Juglans* because of an expression of the dominant *Lyonia*-preference allele. Even if F₁ males mate with *Juglans* females, approximately half of the resulting female backcross offspring will avoid ovipositing on *Juglans* as adults. Therefore, alleles for *Lyonia* preference are sequentially removed from the *Juglans* race.



loci would make removal of the genomic components responsible for the resistance to *Lyonia* from the *Juglans* race more easily, and this possibility should be assessed in future mapping studies.

The present study also indicates that the genetics of host adaptation in *A. transecta* contributes to the reproductive isolation of the two host races. If the females of F_1 hybrids mate with males of the *Juglans* race, the females oviposit all eggs on *Lyonia*, but no backcross larvae can survive on *Lyonia* (Fig. 3). Similarly, even if the F_1 hybrids mate with *Lyonia* females or males, only half of the backcross hybrids would express the recessive trait, resulting in reduced viability on *Lyonia* (Fig. 3). Therefore, hybrids suffer from incongruent phenotypes for preference and performance because of the opposite directions of dominance. This incongruent dominance could be a prime barrier against gene flow between the two host races of *A. transecta*.

Although the genetics of ecological adaptations have received much attention in the study of speciation and species differences¹⁴, the implications of such studies for understanding how ecological speciation occurs are unclear. One reason for this lack of clarity is that empirical data for the genetics of ecological adaptation vary among species (e.g., governed by autosomal loci or sex-linked genes, few or many genes, genes of small or large effect, or genes with dominance, epistatic interactions or no dominance)¹⁵. However, the present results and a growing number of studies have demonstrated that phytophagous insects have different genetic bases between preference and performance with different modes of inheritance^{7,8,16–22}. This implies that hybrids are likely to express different, and often functionally incompatible, phenotypes for preference and performance traits. Indeed, Forister²¹ and Nygren *et al.*²² have revealed that the differences in dominance directions and sex-linkage for preference and performance loci break the correlation between the two traits in F_1 hybrids, respectively. The present study further demonstrates that differences in dominance directions between preference and performance loci lead to ecological incompatibilities in subsequent backcross generations (Fig. 3).

In phytophagous insects, the growing larvae often complete their entire development on a single host plant individual. Hence, a set of genes that function well together on one host is crucial and could be an expected evolutionary outcome^{23,24}. Therefore, hybrids with incompatible host-adaptation genes are likely to be under strong disruptive selection as indicated by the Bateson-Dobzhansky-Muller (BDM) model for postzygotic genomic incompatibilities^{25–28}. Therefore, the different modes of inheritance for selected traits may be an important postzygotic isolation mechanism in phytophagous insects. Although these mechanisms have rarely been emphasized, they may prove to be a major factor promoting host-race formation and consequently the high specialization and species diversity observed in phytophagous insects.

Methods

Moth collection and rearing. I collected mined leaves containing larvae from the host plants, *Juglans ailanthifolia* or *J. regia* and *Lyonia ovalifolia*, and maintained them in the laboratory²⁹. Two sympatric (Sendai, Japan [38°15'N, 140°49'E]) and five allopatric host-associated populations (*Juglans* race: Sapporo [43°07'N, 141°34'E] and Yamagata [38°33'N, 140°49'E]; *Lyonia* race: Okazaki [34°94'N, 137°17'E], Kyoto [35°02'N, 135°79'E] and Kirishima [31°86'N, 130°77'E]; see Supplementary Fig. S1 online) were used for the experiments. All moths used for parental crosses were collected from the field as larvae, together with the leaves on which they were mined. Collected larvae were reared on their respective mined leaves in the laboratory following the methodology described by Ohshima²⁹. All rearing and experimental work was conducted at 25 or 27°C under the photoperiod of a 16:8 L:D cycle with 50–70% relative humidity.

Eclosed adult moths were used for crossing, and all crosses were single-pair crosses. A single pair of virgin moths was transferred with an aspirator to one plastic vial (a 118-mm long, 28-mm diameter centrifuge tube) for pairing. After mating, each female that had started oviposition was individually introduced into a plastic container (120 x 200 x 50, 125 x 225 x 50 or 175 x 260 x 50 mm, depending on the leaf size). Three to six leaves of a host plant were placed in the container to obtain F_1 and backcross hybrid eggs. All F_1 hybrid larvae were maintained on *Juglans* because F_1 hybrid larvae can survive only when they feed on *Juglans* (i.e., resistance to *Juglans* is dominant over that to *Lyonia*)⁹.

The *Juglans* and *Lyonia* leaves used in the present experiments were collected directly from the study localities or from plants that had been transplanted from the localities to greenhouses and randomly used for each trial. I used two *Juglans* species, *J. ailanthifolia* and *J. regia*, for the present experiments, but there were no significant differences in either ovipositing female preference or larval performance between *J. ailanthifolia* and *J. regia* (Ohshima pers. obs.).

Establishment of F_1 hybrids for assessing oviposition preference. The sympatric Sendai populations were used for assessing oviposition preference in F_1 hybrid females. Ten broods were established for the JL F_1 hybrids (crosses between *Juglans* females and *Lyonia* males), and four broods were established for the LJ F_1 hybrids (crosses between *Lyonia* females and *Juglans* males) (see Supplementary Fig. S2A online). Because *Lyonia* females did not normally oviposit on *Juglans* leaves, and it was impossible to transfer eggs to other leaves, I tried to induce the *Lyonia* females to mis-oviposit on *Juglans* leaves by placing a single *Juglans* leaf over *Lyonia* leaves in the plastic container. For this reason, a small number of F_1 adult females were available in the LJ F_1 hybrids (a total of five).

Establishment of F_2 and backcross hybrids for assessing larval performance. First, F_2 and all combinations of backcrosses were established using the sympatric populations in Sendai (see Supplementary Fig. S2B–F online). Five broods were established for the JL x J backcross (crosses between F_1 females from JL crosses and *Juglans* males) (Fig. S2B). Similarly, five broods of the JL x L backcross (crosses between F_1 females and *Lyonia* males) (Fig. S2C), J x JL (crosses between *Juglans* females and F_1 males) (Fig. S2D), L x JL (crosses between *Lyonia* females and F_1 males) (Fig. S2E) and F_2 generations, JL x JL (crosses between F_1 females and males from JL crosses) (Fig. S2F) were prepared. I also prepared JJ (crosses between *Juglans* females and males) and LL (crosses between *Lyonia* females and males) crosses as controls using the Sendai populations (five broods each). Due to the strong host preference of ovipositing females, larvae of backcross hybrids from J x JL and a control cross JJ were exclusively maintained on *Juglans*. Similarly, hybrid larvae from JL x J, JL x L, L x JL, JL x JL (F_2 hybrids), and LL (control) crosses were maintained on *Lyonia* (F_1 hybrid females exclusively prefer to oviposit on *Lyonia*. See the main text). These experiments were conducted from 2003 to 2005.

I further assessed the segregation patterns of larval performance in backcrosses between F_1 hybrid females and *Lyonia* males and vice versa (JL x L and L x JL backcrosses) using three allopatric populations (*Juglans* race: Yamagata; *Lyonia* race: Okazaki and Kirishima). Six and nine broods were established for the JL x L and L x JL backcrosses, respectively (see Supplementary Fig. S2J, K online). These experiments were conducted in 2009.

Establishment of F_2 and backcross hybrids for assessing oviposition preference. For F_2 and backcross hybrids, larvae of each cross combination used in the assessment of larval performance were maintained until adulthood to investigate female oviposition preference, except for JL x J backcrosses (because they are lethal). An additional 22 broods were established for the J x JL backcross, and five broods were established for F_2 hybrids (see Supplementary Fig. S2G, H online). These experiments with the Sendai populations were conducted from 2003 to 2005 and in 2010.

I further assessed the segregation patterns of oviposition preference using a J x JL backcross that originated from Sapporo (*Juglans* race) and Kyoto (*Lyonia* race) populations. Thirteen broods were established for these crosses (see Supplementary Fig. S2I online) in 2007.

Assessing larval performance in F_2 and backcross generations. A previous study revealed that hybrid larvae with a maladaptive performance genotype could not survive until the second stadium on *Lyonia*⁹. For this reason, I recorded the numbers of hatched eggs and the viability of larvae that reached the second stadium as an index of larval performance.

There was no observed heterogeneity of segregation patterns among broods within a given category of mating (G-test, JL x JL F_2 [Sendai] $G = 6.995$, $P = 0.1361$, $df = 4$; JL x J backcross [Sendai] $G = 0$, $P = 1$, $df = 4$; JL x L backcross [Sendai] $G = 5.497$, $P = 0.2399$, $df = 4$; J x JL backcross [Sendai] $G = 7.624$, $P = 0.1063$, $df = 4$; L x JL backcross [Sendai] $G = 2.839$, $P = 0.5850$, $df = 4$; JL x L backcross [Yamagata x Okazaki x Kirishima] $G = 8.912$, $P = 0.1126$, $df = 5$; L x JL backcross [Yamagata x Okazaki x Kirishima] $G = 1.529$, $P = 0.9922$, $df = 8$). Therefore, the results are presented for pooled data.

Assessing oviposition preference in F_1 , F_2 and backcross generations. Eclosed F_1 , F_2 and backcross females were crossed with males from their own generation (e.g., F_2 females crossed with F_2 males) for testing host preference, and all crosses were single-pair crosses. A single pair of virgin moths was transferred with an aspirator to one plastic vial (a 118-mm long, 28-mm diameter centrifuge tube) for pairing. After mating, each female that had started oviposition was individually introduced into a plastic container (100 x 100 x 50 mm). One pair of fresh young leaves of *Juglans* and *Lyonia* were placed in the container. Similar-sized leaves were chosen. In this container, ovipositing females were allowed to select leaves for oviposition for 24 h after transfer, and the number of eggs deposited on each leaf was counted.

There was no observed heterogeneity of segregation patterns among broods within a given category of mating (G-test, JL F_1 $G = 4.803$, $P = 0.8511$, $df = 9$; LJ F_1 $G = 0$, $P = 1$, $df = 3$; JL x JL F_2 [Sendai] $G = 0$, $P = 1$, $df = 9$; JL x L backcross [Sendai] $G = 0$, $P = 1$, $df = 4$; J x JL backcross [Sendai] $G = 28.86$, $P = 0.3174$, $df = 26$; L x JL backcross [Sendai] $G = 0$, $P = 1$, $df = 4$; J x JL backcross [Sapporo x Kyoto] $G = 13.21$, $P =$



0.3539, $df = 12$). Therefore, results are presented for pooled data. Females were considered to be a *Juglans* (or *Lyonia*) type if more than half of the eggs they laid were on *Juglans* (or *Lyonia*).

Statistical analyses. Egg hatchability and larval viability until the second stadium of the F_2 , backcross and control generations were transformed to arcsine square roots to satisfy the requirement of normality, and the transformed values were analyzed using one-way ANOVA. For viability, a significant difference was found among crosses ($F_{8,41} = 82.09$, $P < 2.2e-16$). Thus, post hoc Tukey-Kramer HSD ($\alpha = 0.05$) pairwise comparisons were performed between crosses. The numbers of eggs deposited on the respective leaves in the oviposition preference experiments were analyzed by a paired t -test. The hypotheses of a single-locus, two-allele system with complete dominance for the segregation patterns of larval performance and of ovipositing female preference in F_2 and backcross generations were analyzed by goodness-of-fit (G -test)³⁰. All statistical tests were performed using R 2.11.0³¹.

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Author contributions

I.O. conceived and designed the study, collected materials, reared moths with the assistance of those mentioned in the Acknowledgements, collected data, analyzed the data and wrote the paper.

Additional information

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