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The evolution of haplodiploidy by male-killing endosymbionts: importance of population structure and endosymbiont mutualisms

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Abstract

Haplodiploid inheritance systems, characterized by male transmission of only their maternally inherited genomic elements, have evolved more than 20 times within the animal kingdom. A number of theoretical studies have argued that infection with certain male-killing endosymbionts can potentially lead to the evolution of haplodiploidy. By explicitly investigating the coevolutionary dynamics between host and endosymbiont, we show that the assumptions of current models cannot explain the evolution of haplodiploidy very well, as the endosymbiont will often go extinct in the long term. Here, we provide two additional mechanisms that can explain the stable evolution of haplodiploidy by male-killing endosymbionts. First of all, a spatially structured population can facilitate the long-term persistence of haplodiploidy, but this applies only when levels of inbreeding are very high. By contrast, endosymbionts that are mutualistic with their hosts provide a much more general and promising route to the stable evolution of haplodiploidy. This model is the first to provide a formal explanation of the supposed association between the evolution of haplodiploidy and the highly inbred lifestyles of some ancestors, while it also provides a hypothesis for the evolution of haplodiploidy in more outbred ancestors.

Introduction

Haplodiploidy is a genetic system in which males transmit exclusively maternally inherited genes to the next generation. Arrhenotokous haplodiploidy (defined as the development of unfertilized haploid eggs into males and fertilized diploid eggs into females) is well known from groups such as the Hymenoptera and Thysanoptera. Haplodiploidy also comprises paternal genome elimination (PGE), in which the paternally inherited genome is eliminated from diploid male eggs. PGE is a common mode of inheritance in groups such as the scale insects (Iceryini, Neococcoidea) and sciarid flies (Sciaridae) (Hughes-Schrader, 1948; Haig, 1993). Haplodiploidy in both forms has evolved at least 20 independent times, 10 of which in insects (Otto & Jarne, 2001; Normark, 2003, 2004a). It is currently poorly understood why haplodiploidy has evolved only in some groups and not in others. A number of different hypotheses have been postulated on the adaptive significance of haplodiploidy.

Maternal transmission advantage. All of the genes transmitted by haploid sons are of maternal origin, thereby partially circumventing the twofold cost of sex (Brown, 1963, 1964). As noted by Normark (2004a), this advantage of haplodiploidy over diplodiploidy always holds true from the maternal perspective. However, it fails to explain why haplodiploidy is only found in specific groups of organisms.

Deleterious mutation clearance. Deleterious mutations can be purged more efficiently when there is an extensive haploid male phase (Goldstein, 1994). Again, clearance of deleterious mutations can be regarded as an inherent advantage to haplodiploidy and therefore fails to explain its particular phylogenetic distribution. For example, haplodiploidy as an adaptation to mutagenic or exposed environments does not match current ecological data on haplodiploid ancestors (Bell, 1982; Normark,

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2004a). Furthermore, this hypothesis would not apply to certain forms of PGE in which the paternally inherited genome is still expressed in many tissues (as is the case in *Sciara*, Goday & Esteban, 2001 or in the lecanoid and diaspidid PGE systems in scale insects, Herrick & Seger, 1999).

Maternal sex ratio control. Arrhenotokous haplodiploidy facilitates maternal control of the sex ratio, and it may thus be favoured when such maternally controlled sex ratios are selectively favoured, which can be the case under certain conditions of inbreeding (Hamilton, 1967; Borgia, 1980; Charnov *et al.*, 1981). Although this hypothesis matches with the inbred life histories of many haplodiploid ancestors, it fails to provide a mechanistic explanation for the transition between diplodiploidy and haplodiploidy. Also note that this hypothesis only applies to systems exhibiting PGE when some maternal control on the elimination of paternally inherited genome can be assumed.

Endosymbiont-induced haplodiploidy. A more mechanistic extension of the previous hypothesis on sex ratio control was provided by W.D. Hamilton, based on the observation that inbred haplodiploid groups such as bark beetles and mites are infected by endosymbiotic bacteria. Hamilton postulated that endosymbionts present in heterogametic males (XY) of a diplodiploid ancestor may have eliminated or disabled the paternally inherited chromosome set, allowing only the transmission of the maternal, X-bearing chromosome set to offspring, which results in an overproduction of daughters as offspring. Especially under conditions of inbreeding, such higher proportions of daughters would be strongly favoured by both the endosymbiont and the maternal host (Hamilton, 1978, 1993). In contrast to the previous two hypotheses, this hypothesis appears to focus more on the evolution of PGE than on arrhenotokous haplodiploid systems, as it still assumes fertilization of an egg by the paternal genome, after which elimination takes place.

Recently, Hamilton's idea about the role of endosymbionts in the evolution of haplodiploidy saw renewed interest after a meta-analysis indicated that other haplodiploid ancestors are also associated with maternally inherited endosymbionts (Normark, 2003). Moreover, almost all of the haplodiploid ancestors appear to have an increased scope for kin competition, as broods are gregarious and interact in a confined space such as crevices or bark galleries. On the basis of supposed preponderance of both endosymbionts and gregarious broods, Normark (2004a) extended Hamilton's hypothesis by assuming an endosymbiont with a male-killing phenotype that would haploidize males. Male-killing endosymbionts are associated with gregarious broods, as these endosymbionts can only persist when enough resources are reallocated from killed males to their infected female sibs (Werren, 1987; Hurst, 1991; Freeland & McCabe, 1997).

The hypothetical endosymbiont in Normark's model achieves male killing by elimination of the incoming paternal genome upon fertilization, when this genome carries a male-determining element (i.e. a Y chromosome). This renders male zygotes haploid and therefore inviable. Central to Normark's hypothesis is that some haploidized males survive this haploidization and may eventually evolve towards normal levels of survival. Normark showed that haplodiploidy according to this hypothesis would evolve in a relatively wide range of values of both resource reallocation efficiency and haploidized male viability (Normark, 2004a). More sophisticated analyses by Engelstädter & Hurst (2006) and Ubeda & Normark (2006), which also took into account sex ratio selection, showed that the endosymbiont is capable of persisting whenever the product of endosymbiont transmission rate a and the average offspring survival R is larger than 1 (see Table 1 for summary of main notation used). However, neither of these studies investigated whether coevolution between host and endosymbiont can lead to long-term persistence of haploidizing endosymbionts. In this study, we demonstrate that current models in fact do not allow for a long-term persistence of haplodiploidy by male haploidizing endosymbionts and therefore do not provide a satisfactory explanation for the evolution of haplodiploidy. We show that additional ecological features of haplodiploid ancestors, such as spatial population structure and direct mutualistic benefits provided by the endosymbiont, are required for the long-term persistence of haplodiploidy.

Table 1 A summary of the main notation used in the text.

Variable	Description
a	Endosymbiont transmission probability of mutant foundress
a^*	Endosymbiont transmission probability of resident foundress
k	Number of males and females produced per foundress
s	Survival probability of a focal haploidized male
$\bar{s}$	Survival probability of a haploidized male sharing a brood with the focal male
$\tilde{s}$	Survival probability of a haploidized male sharing a patch with the focal male
s^*	Survival probability of a resident haploidized male
b	Efficiency of resource redistribution from sons to siblings
$n_f^{i,u}$	Number of infected 'i' or uninfected 'u' females
$n_m^{i,u}$	Number of infected 'i' (haploidized) or uninfected 'u' (diploid) males
$x_m^{i,u}$	Equilibrium class frequency of infected 'i' or uninfected 'u' males
$x_f^{i,u}$	Equilibrium class frequency of infected 'i' or uninfected 'u' females
$v_m^{i,u}$	Reproductive value of infected 'i' or uninfected 'u' males
$v_f^{i,u}$	Reproductive value of infected 'i' or uninfected 'u' females
p_1^*	Average numbers of mates of an uninfected male
p_2^*	Average numbers of mates of an infected male
m	Direct host survival benefit of possessing endosymbiont
N	Number of foundresses per patch

Spatial population structure can have important consequences for the evolutionary dynamics of endosymbiont-induced haplodiploidy (EIH). On the one hand, it may promote the coexistence of endosymbionts and their hosts, as female-biased sex ratios caused by a sex ratio-distorting endosymbiont may benefit the host under conditions of local mate competition. Furthermore, local extinction–recolonization dynamics can stabilize coexistence between hosts and sex ratio distorters, such as male-killing endosymbionts (Hatcher *et al.*, 2000; Groenenboom & Hogeweg, 2002). On the other hand, spatial population structure may also hamper the coexistence of endosymbionts and their hosts because inbreeding can devalue the maternal benefit of having haploidized sons, as inbreeding reduces the relative increase in relatedness of a mother to her haploid over her diploid sons (Smith, 2000). Given these opposing selection pressures, it is difficult to predict without a formal analysis whether or not spatial population structure facilitates the evolution of haplodiploidy.

In addition, the type of symbiosis between the host and the endosymbiont may have been an important factor affecting the coevolution of a haplodiploid ancestor with its endosymbiont. Maternally inherited endosymbionts can vary from being entirely parasitic manipulators of their host's reproductive systems, to having more mutualistic relationships in which the host accrues certain benefits from being infected (e.g. through provision of nutrients or protection against stress), extending even to relationships that are fully obligate and in which hosts are unable to reproduce without endosymbiont infection (Moran *et al.*, 2008). The previous models on the evolution of haplodiploidy considered male-killing endosymbionts that did not confer any direct benefits upon their host. Here, we generalize these models by allowing for varying degrees of such direct benefits and investigate the effect of this on the long-term persistence of haplodiploidy.

In this paper, we use individual-based simulations in combination with an analytical kin selection model to examine the significance of spatial population structure and endosymbiont mutualisms for the evolution of haplodiploidy. In contrast to previous models that rely on invasion analyses, we used individual-based simulations in addition to an analytical reproductive value approach to examine the full coevolutionary dynamics between the host and the endosymbiont.

The model

The main goal of our model was to investigate the invasion prospects as well as the long-term persistence of an endosymbiont with a male haploidizing phenotype, in an initially diplodiploid population. The endosymbiont is transmitted vertically by a maternal host to her offspring with transmission probability a. Initially, the offspring have a 1 : 1 sex ratio. Males that are infected by the endosymbiont are haploidized during early development. A proportion s of haploidized males are assumed to survive. A specific scenario would be that such surviving males have mutations in a pre-existing dosage compensation complex, so that it upregulates expression in a haploidized male to match expression levels of a diploid male. If a male does not survive haploidization, his resources are reallocated to the remaining members of the brood with efficiency factor b. This means that a proportion b of the resources allocated to such males will become available to their surviving sibs. Specifically, the relative amount of resources available to survivors is given by:

$$R = 1 + \frac{ba(1-s)}{2 - a(1-s)}. \qquad (1)$$

This equation shows that offspring of uninfected females have a baseline amount of resources of 1, whereas offspring of infected females receive an additional $ba(1-s)$ units of resources from brothers that did not survive haploidization, equally shared over the $2 - a(1-s)$ surviving sibs.

Nonspatial model

Invasion condition for haplodiploidy

We first describe our analytical framework by formulating a nonspatial version of the model, which is based on previous models by Engelstädter & Hurst (2006) and Ubeda & Normark (2006) but uses a reproductive value approach (Taylor, 1996; Pen & Weissing, 2002). In Appendix A, we generalize previous models by adding the possibility that the endosymbiont can also provide direct benefits to its host. Specifically, an infected host has $1 + m$ times the amount of resources of an uninfected host. Thus, we obtain a more general condition for the persistence of a male haploidizing endosymbiont:

$$(1+m)aR > 1 \qquad (2)$$

Mutant invasion dynamics

Following Ubeda & Normark (2006), control of a and s was given to either the host or the endosymbiont, resulting in four different coevolutionary scenarios (see Appendix B and Fig. 1). In this analysis, we did not assume any direct benefits of being infected with the haploidizing endosymbiont. The results are summarized in Fig. 1: if the endosymbiont is in control of the survival of haploidized males s, selection favours maximal resource reallocation to daughters, leading to complete mortality of infected males and either extinction of the population as a whole or the complete loss of the endosymbiont from the population (Fig. 1b,d). Therefore, invasion and successful short-term persistence of the endosymbiont in a population of hosts is only possible when the host is in control of s. In that case,

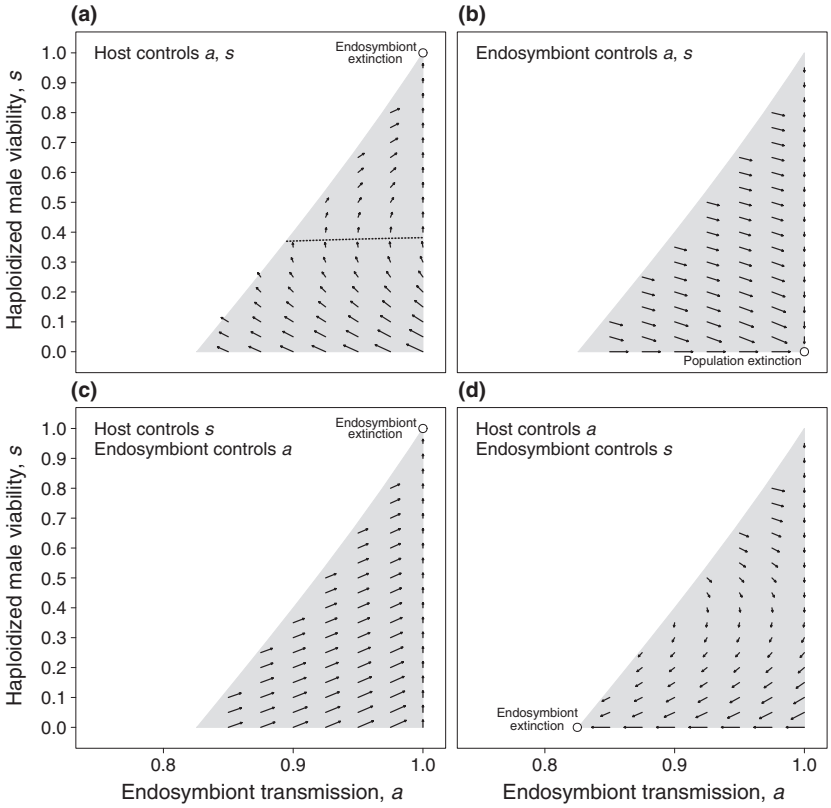

Fig. 1 Coevolutionary dynamics of endosymbiont transmission a and haploidized male viability s in the nonspatial model, under different scenarios of host and endosymbiont control. The grey area depicts the parameter space in which condition (2) is met, which allows persistence of the endosymbiont. In all four cases, evolution between host and endosymbiont leads to extinction of the endosymbiont. (a) Host control of both a and s. When s is still too low, females suffer from being infected with the endosymbiont and are selected for lower levels of a. This can lead to evolution outside the grey area and thus extinction of the endosymbiont. The dotted black line depicts the minimal value of s beyond which the host is selected to favour higher endosymbiont transmission rates, as at that point the maternal transmission advantage of the s viable haploidized sons outweighs the deaths of the $1 - s$ remaining males. Beyond that line, a and s both evolve towards 1. At the point $\{a,s\} = \{1,1\}$, no resources are reallocated to the endosymbiont, and the endosymbiont goes extinct due to drift. (b) Endosymbiont control of both a and s. s is selected towards zero, and transmission rates a evolve towards 1, after which the population goes extinct due to lack of males. (c) Endosymbiont control of a, host control of s. The endosymbiont will always be selected to increase transmission a and the host is selected to increase s as well. Nevertheless, at the point $\{a,s\} = \{1,1\}$ the endosymbiont again goes extinct, as resource reallocation to infected hosts ceases. (d) Host control of a, endosymbiont control of s. s and a are both always selected against, leading to extinction of the endosymbiont. Parameters: $b = 0.3$, $m = 0$.

the maternal host may benefit from the endosymbiont because viable haploidized sons are more efficient vehicles for her genes compared with diploid sons. Selection will then favour ever-increasing haploidized male viability s as well as endosymbiont transmission rates, regardless of who controls the latter (Fig. 1a,c). This will continue until both s and a reach their maximal values of unity. These results were also obtained by Ubeda & Normark (2006). However, these authors did not point out that long-term persistence of the haploidizing endosymbiont is not possible in this equilibrium. The reason is that maximal survival of haploidized males implies that no reallocation of resources to daughters occurs ($R = 1$), in which case inequality (2) is no longer satisfied and the endosymbiont will drift to extinction. Additional mechanisms are thus needed to allow long-term persistence of the haploidizing endosymbiont in the population. Below, we investigate the role of spatial population structure as well as the role of direct benefits conferred by the endosymbiont upon its host.

Spatial model

Life cycle

A population consists of 4000 initially diploid individuals and is subdivided into $2000/N$ identical patches, in which N is the number of foundresses per patch. To investigate the effect of inbreeding, N was varied: $N = \{2, 5, 10, 20, 50\}$. Each foundress produces 20 eggs and the sex of each egg is randomly assigned. If the foundress carries male

haploidizing endosymbionts, each egg has a chance a of being infected, where a is determined by a single locus with many potential alleles and is either under maternal (diploid) or endosymbiont (haploid) control. At the start of each simulation the population was monomorphic for a specific transmission rate $a_{t=0}$ which was varied between simulations in the range from 0 to 1. Initially, 10% of the population was infected by the endosymbiont. After male killing took place in a brood, resources were reallocated from killed males to their siblings according to the formula for R given in eqn 1. Subsequently, both sexes undergo resource-based survival, in which their survival probability is given by $\frac{1}{2}R$.

Following resource-based survival, females mate in their natal patch with a random male (allowing for sib mating). If no fertilization opportunities are present because no males survived the male killing or the resource-based survival phase, the patch goes extinct. All fertilized females are added to a dispersal pool, from which the foundresses of the new generation are randomly selected to found a random patch. The mutation rates for a and s were fixed at 0.01 and the mutation steps were taken from a normal distribution with mean of 0 and standard deviation of 0.01. We discuss only the results when the host was in control of s, as simulations of our spatial model when the endosymbiont was in control of s were similar to the nonspatial version and therefore showed no long-term endosymbiont persistence. This agrees with the result of Groenenboom & Hogeweg (2002), who found endosymbiont persistence to occur only in spatially explicit models and not in spatially implicit models having random dispersal, which is similar to the approach we used.

Results

In a scenario in which either the host or the endosymbiont has control of endosymbiont transmission, stable persistence of the haploidizing endosymbiont is generally not possible. Figure 2 depicts the persistence of the endosymbiont when the host is in control of its transmission: endosymbionts are generally unable to be maintained in the population for a period that is longer than 20 000 generations. Figures S1 and S2 show 10 replicate runs of such simulations over time, in the case of, respectively, host or endosymbiont control of a. The latter figures illustrate that the initial endosymbiont invasion is followed by ever-increasing numbers of viable haploidized males, after which the endosymbiont goes extinct again due to lack of resource reallocation, thereby restoring the initial diplodiploid population. The purging of the endosymbiont and the resulting loss of haploidized males for almost all values of $a_{t=0}$ and b in our simulations confirm our previous results from the nonspatial model that, although transient coexistence of host and endosymbiont may be possible in the short term, persistence of the endosymbiont and haploidized males is not possible in the long term.

Figure 2, however, also shows that in the case of $N = 2$, the endosymbiont is sometimes capable of persisting for longer than 20 000 generations. Although extended simulations show that also in these cases, the endosymbiont will eventually go extinct before generation 30 000, a kin selection model (Appendix S1) shows that $N = 2$ represents a boundary case of a region of very high local relatedness in which long-term endosymbiont persistence is possible. When relatedness is high, local

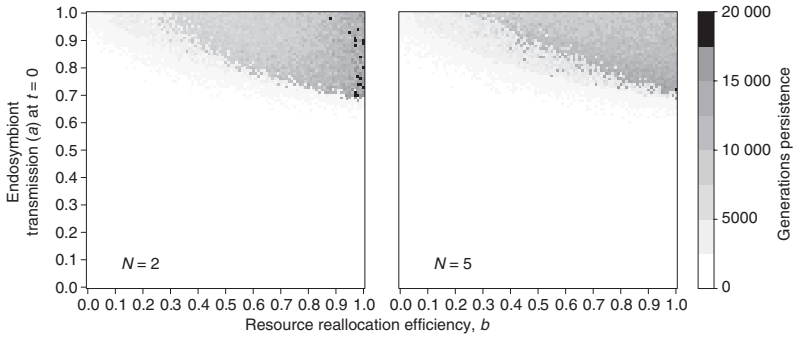

Fig. 2 No stable persistence possible of the male haploidizing endosymbiont in the spatial model under female-biased dispersal, when the host is in control of both endosymbiont transmission probability a and haploidized male viability s. The shade of each cell represents the persistence (in generations) of the haploidizing endosymbiont during a single simulation run (for example simulations, see Fig. S1). Each run is characterized by an initial transmission probability $a_{t=0}$ (y-axis) and resource reallocation efficiency b (x-axis) for two different patch sizes $N = 2$ and $N = 5$. Results for $N = \{10,20,50\}$ resemble $N = 5$ and are therefore not shown. The male haploidizing endosymbiont will only persist for 10 000 generations or less. Only when inbreeding is very common, endosymbiont persistence and the presence of haploidized males is continued for longer than 20 000 generations (left panel) but also in these cases the endosymbiont will eventually go extinct (results not shown). $N = 2$ represents a boundary case of long-term persistence of the male haploidizing endosymbiont, when relatedness is very high (see Supporting information). In this figure, the host did not accrue any additional benefits from the endosymbiont, $m = 0$.

mate competition will select haploidized males to forego mating themselves. Instead, a certain percentage of males now allow themselves to be killed, so that their resources can be reallocated to their dispersing sisters. In this scenario, optimal levels of s will thus be below 1, in which case $R > 1$ and therefore condition (2) remains satisfied. Thus, a spatial population structure can in principle allow for the stable persistence of haplodiploidy, but only under very high levels of inbreeding (see also Fig. S3).

Endosymbiont mutualisms

We can conclude from the previous sections that stable persistence of both the haploidizing endosymbiont and viable haploidized males is virtually impossible, unless inbreeding is extreme. However, the previous analysis only took into account a purely parasitic relationship of the endosymbiont with its host, whereas many cases exist in which an endosymbiont provides a competitive advantage to its host through means other than resource reallocation of killed males. An increasing number of examples are reported of endosymbiont infections in which the endosymbiont provides certain nutritional functions to its host (Moran *et al.*, 2008).

We incorporated a scenario of endosymbiont mutualism in the nonspatial model by allowing for a slight benefit of endosymbiont infection: $m = 0.05$ (see Fig. 3). A possible scenario corresponding to such a value of m would be that the male haploidizing endosymbiont provides a certain nutrient to its host, but the host enjoys only a slight advantage from this (our model could consider obligate relationships between hosts and symbionts as well, when $m \to \infty$). When the host is in control of s, Fig. 3 shows that $m > 0$ precludes extinction of the endosymbiont when the equilibrium $\{s,a\} = \{1,1\}$ is attained: direct benefits always provide infected hosts with a competitive advantage over uninfected hosts, which allows the endosymbiont to be maintained in the long term, even if resource reallocation from dead haploidized males ceases when s attains 1. We can thus conclude that even slight amounts of direct benefits of endosymbiont infection assure long-term persistence of the male haploidizing endosymbiont.

To check if these conclusions also apply to a spatial context, we ran simulations of our spatial model for three different values of endosymbiont survival benefits: $m = \{0.05, 0.11, 0.25\}$. Again, Figs 4 and 5 show that the incorporation of small survival benefits drastically alleviates the restrictive conditions under which the haploidizing endosymbiont can stably persist; the endosymbiont is already maintained under modest values of $a_{t=0}$ and b and although the degree of local relatedness may increase the likelihood of endosymbiont persis-

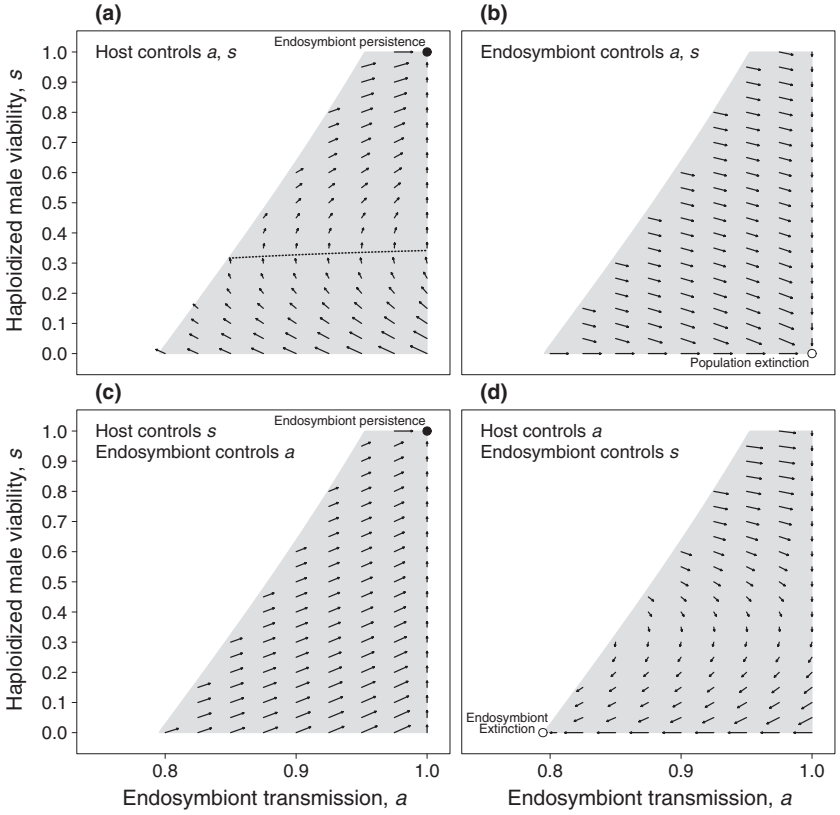

Fig. 3 Coevolutionary dynamics of endosymbiont transmission a and haploidized male viability s in the nonspatial model, when the male haploidizing endosymbiont confers direct benefits ($m = 0.05$) upon its host. Direct benefits now allow for persistence of the endosymbiont, when the host is in control of s. (a,c) A larger region now exists in which condition (2) is satisfied, which is especially important when coevolution has reached point $\{a,s\} = \{1,1\}$. Direct benefits now maintain the endosymbiont's advantage in comparison with uninfected hosts, despite the lack of resource reallocation. Direct benefits do not alter the conclusions when the endosymbiont is in control of s: either extinction of both the host and endosymbiont occurs (b) or only that of the endosymbiont (d): the endosymbiont still favours $s = 0$, leading to extinction of either the host population or only the endosymbiont. Parameters: $b = 0.3, m = 0.05$.

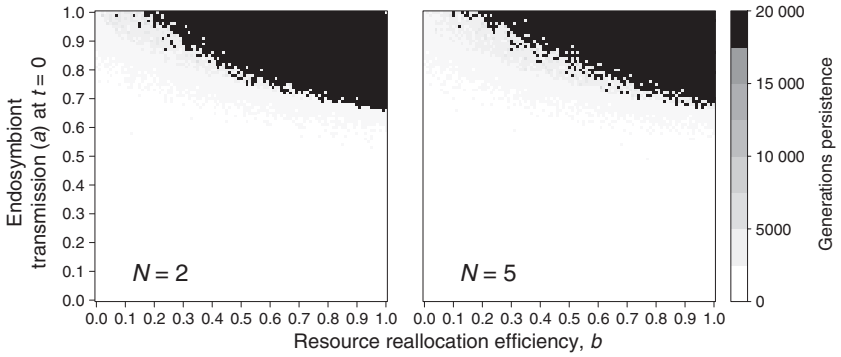

Fig. 4 Small direct viability benefits from carrying the male haploidizing endosymbiont leads to stable persistence of haplodiploidy. As in Fig. 2, the host is in control of both haploidized male viability s and the endosymbiont transmission rate a, but now the host has a direct viability advantage of 5% of carrying the endosymbiont ($m = 0.05$). Results for $N = \{10,20,50\}$ resemble $N = 5$ and are therefore not shown. For details, see Fig. 2.

tence even further, it is not a necessary requirement for long-term stability. From both a spatial and a nonspatial version of our model, it can be concluded that even small degrees of endosymbiotic benefits can stabilize persistence of the male haploidizing endosymbiont.

Discussion

Three recent models explored the conditions under which endosymbionts with a male haploidizing phenotype could lead to the transition from diplodiploidy to a haplodiploid genetic system (Normark, 2004a; Engelstädter & Hurst, 2006; Ubeda & Normark, 2006). The general conclusion of these models was that EIH could in principle evolve, but only under rather restrictive conditions including high endosymbiont transmission and high levels of resource reallocation. Our analysis shows that achieving permanent haplodiploidy under the assumptions used in previous models is in fact not possible: scenarios that allow for the invasion of the haploidizing endosymbionts and viable haploidized males will eventually also select for maximal levels of haploid male viability. As soon as all males survive from haploidization, resource reallocation to infected hosts ceases, thereby eliminating any fitness benefits for hosts infected with the endosymbiont and making the endosymbiont very likely to be purged due to drift effects. In order to achieve a permanent maintenance of EIH, additional mechanisms have to be assumed that maintain a permanent fitness advantage of infected over uninfected hosts.

Both this study and previous studies have not addressed scenarios in which control of haploidized male viability or endosymbiont transmission is 'shared' in some fashion between host and endosymbiont. The simplest scenarios of such joint control would be when gene products of host and endosymbiont interact either additively (e.g. phenotype is determined by the total amount of gene products present) or multiplicatively (e.g. gene products of the endosymbiont directly eliminate gene products of the host). In the case of additive control of haploidized male viability (s) one can easily imagine stable coexistence of host and endosymbiont: the optimal endosymbiont's viability level is $s_e = 0$, whereas the host's optimum is $s_h = 1$, leading to an average survival probability of $\bar{s} = 0.5$ in a haploidized male individual, which would lead to long-term coexistence (see eqn 2). As this scenario is optimal neither for the host nor for the endosymbiont, it is likely to be prone to invasion by a modifier which either bypasses the currently used pathway, leading to full control of one party and eventually resulting in a scenario described in this and previous studies. In a simple multiplicative scenario, evolution of both loci would also lead to one party winning the conflict, as now $\bar{s} = s_e s_h = 0 \times 1 = 0$. To conclude, an important question left for future studies is to what extent more complex scenarios of interaction (i.e. multiple loci or specific genetic constraints) are capable of preventing one party winning the conflict or at least prolong intermediate coexistence for a considerable time.

In this study, we investigated two different, but not mutually exclusive, routes that may lead to a situation in which a competitive advantage of infected hosts over uninfected hosts is maintained. First of all, by assuming a spatially substructured population with female-biased dispersal in which mothers produce a 1 : 1 sex ratio, we showed that high levels of relatedness between random males and females within a deme may be sufficient to achieve stable haplodiploidy. As soon as the offspring of less than two foundresses compete on a patch, it can be worthwhile for a male to allow itself to be killed by the endosymbiont, to reallocate his resources to the sisters in his brood that disperse. When relatedness or resource reallocation efficiency is too low, a male is better off pursuing matings with other females on the patch and

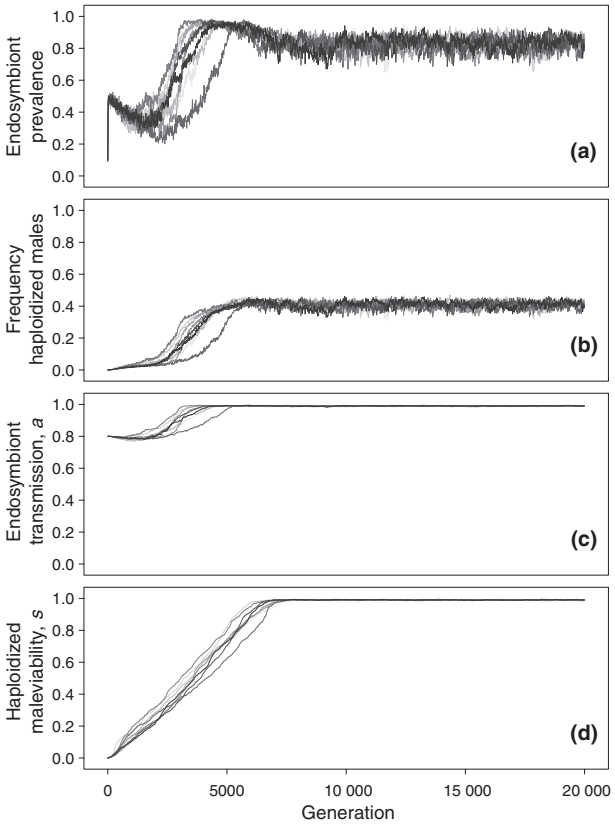

Fig. 5 Ten replicate simulations in which male haploidizing endosymbionts are capable of persisting, as they constitute a direct survival benefit to their hosts. (a) The total frequency of the endosymbiont in the population, (b) the frequency of haploidized males in the population, (c) the average endosymbiont transmission rate a under host control and (d) the average survival probability s under host control. Parameters: $b = 0.8$, $a_{t=0} = 0.8$, $m = 0.05$, $N = 20$.

will be selected to maximize his survival probability. If the mother is capable of producing a female-biased sex ratio without the action of the endosymbiont, this would preclude the evolution of haplodiploidy through inbreeding, as the very few males that are produced comprise insufficient resources for the endosymbiont to reallocate. However, strongly female-biased sex ratios in diploid species are generally rare and would require additional assumptions such as gamete selection (Reiss, 1987; Pen & Weissing, 2002). The strong dependence of the evolution of haplodiploidy on the level of local relatedness closely matches Hamilton's predicted association of inbreeding and female-biased sex ratios with haplodiploid ancestors (Hamilton, 1967). Our study is the first formal model that explicitly links the presence of endosymbionts and the inbred lifestyle of many of these haplodiploid ancestors with the actual evolution of haplodiploidy.

A second route to haplodiploidy is when the competitive advantage of infected over uninfected hosts is realized by means other than resource reallocation (Hurst *et al.*, 1997). Endosymbionts can provide important functions for the host's nutrition, as demonstrated by numerous cases of endosymbionts that are involved in nutrition (Dale & Moran, 2006; Janson *et al.*, 2008; Moran *et al.*, 2008), or play a role in the host's reproduction (Peleg & Norris, 1972; Starr & Cline, 2002; Zchori-Fein *et al.*, 2006). By giving infected hosts a small survival advantage, we demonstrated that male haploidizing endosymbionts can persist across a much larger range of parameters, as their hosts always have a competitive edge over uninfected hosts, irrespective of potential resource reallocation. This also reduces the dependence of the male haploidizing endosymbiont on high levels of local relatedness: cessation of resource reallocation due to the complete rescue of haploidized males when $N \geq 2$ may reduce some part of the competitive advantage, but the direct survival benefit m maintains the haploidizing endosymbiont in the population.

To conclude, EIH through the mechanism investigated in this study is likely to evolve through two different routes. The first route requires that four conditions are met, namely high transmission fidelity of the haploidizing endosymbionts, high levels of resource reallocation, extremely high relatedness and a sex chromosome system that prevents mothers from producing female-biased sex ratios autonomously. The second route to haplodiploidy appears to be more general: it requires that the endosymbiont bestows direct benefits on its host, accompanied by minimally modest levels of endosymbiont transmission fidelity and efficiency of resource reallocation. We will now briefly address the empirical evidence on whether these conditions are likely to be met.

First, a key assumption of the EIH is the putative mechanism of male haploidization: that the endosymbiont detects the incoming male genome that carries a Y, and eliminates it before zygote development is fully initiated. Investigations into the molecular basis of male detection showed that male-killing *Spiroplasma* that infect *Drosophila* detect maleness based on specific proteins of the male dosage compensation complex (Veneti *et al.*, 2005). Killed males have intact germline formation and only somatic cells are affected, which is not in line with the EIH hypothesis. Recently, a different male-killing mechanism that acts at a much earlier stage of development has been found in the haplodiploid wasp *Nasonia*. Here, *Arsenophonus* bacteria blocked centrosome formation, thereby deregulating the first nuclear division of males (Ferree *et al.*, 2008). Nevertheless, this cytologically appealing mechanism of male killing is still confined to haplodiploids, in which ploidy differences between the sexes make cytological detection of males vs. females potentially much more straightforward than in any diplodiploid ancestor. Our hypothesis would require a male-killing endosymbiont that: (i) could detect maleness before germline differentiation based on sex

chromosome content or other sex-specific cytological cues, and (ii) is capable of eliminating the paternally inherited genome copy as a whole, potentially by targeting the formation of the paternally inherited centrosome. To what extent such a mechanism is plausible can only be investigated by assessing the cytological mechanisms that are used by other known male-killing endosymbionts, for example the different types of bacteria that are present in ladybirds (Hurst *et al.*, 1997). More information on potential idiosyncrasies in the cytogenetic machinery of haplodiploid ancestors may reveal why inheritance systems in certain clades appear to be much more vulnerable to endosymbiont action than in others.

Related to the previous point on the detection of diploid males by the endosymbiont is the subsequent assumption of EIH that haploidized individuals are always transformed into males (Ubeda & Normark, 2006). This is especially problematic, as the genome containing a genetic element that is always associated with males (i.e. containing the Y chromosome) is assumed to be eliminated in our model. Moreover, in the insect model system *Drosophila*, haploid mutants develop as females and not as males. However, an important thing to note from the sex-determining cascades of *Drosophila* and other insects like *Musca* is that female development requires that the main protein on top of the sex-determining cascade successfully achieves a self-regulatory feedback loop (e.g. *sxlPe* in *Drosophila*), whereas male development starts when this feedback loop cannot be instantiated (Cline & Meyer, 1996; Burghardt *et al.*, 2005). When expression levels of these proteins on top of the cascade are thus disrupted during early development (e.g. expression levels are reduced due to haploidization), one may expect male instead of female development. In fact, the reason why haploid mutants do not develop as male in *Drosophila* is that an additional precellular cell division leads to a longer time of sensitivity to *sxlPe* which thus initiates female development in haploid embryos, despite the lower levels of *sxlPe* expression from the haploid genome (Erickson & Quintero, 2007). To what extent haploid mutants of other insects may lack such additional embryonal characteristics and therefore could be prone to male development remains an open question. In any case, explicit modelling of the underlying sex-determining cascade in models that investigate the evolution of haplodiploidy may be necessary to assess which developmental mechanisms increase the likelihood of haploid individuals to develop as males. Furthermore, making explicit assumptions about the mechanism of sex determination is important in the light of the maternal sex allocation decision. If the mother is not constrained by a chromosomal sex-determining system to produce even sex ratios, female-biased sex ratios under local mate competition would make it less beneficial for sons to allow male killing. However, it is an important question to what extent genetic sex determination allows the production of such biased sex ratios, without assuming additional mechanisms such as gamete selection (Krackow, 2002) or, as is our focus here, male killing in combination with intermediate levels of viability as a first step towards the evolution of haplodiploidy. Although our model aims to provide an explanation for the elimination of the paternally inherited genomes in males (PGE), it does not yet give a full account for the evolution of arrhenotokous haplodiploidy, which involves the development of viable haploid males from unfertilized eggs. We follow the conventional viewpoint that PGE can be considered a precursor to arrhenotoky (Cruickshank & Thomas, 1999; Normark, 2004a) and that our mechanism on the evolution of PGE may be followed by other adaptations regarding facultative fertilization of eggs, resulting in arrhenotoky. We note, however, that it is currently debated to what extent PGE can be considered to be a primitive form of arrhenotokous haplodiploidy or if both instances of haplodiploidy have evolved independently or even that PGE may be a derived form of arrhenotoky (Burt & Trivers, 2006; Normark, 2009). In that case, it remains to be seen if our model on EIH applies also to the ancestral form of arrhenotokous haplodiploidy.

The second condition regards the type of relationship that endosymbionts have with their hosts (parasitic or mutualistic). Cases of infection with endosymbionts are present in all the insect haplodiploid ancestral groups, varying from endosymbionts that are strictly obligate to their host to presumably more transient and parasitic interactions involving endosymbionts such as *Wolbachia* (Normark, 2004a and references therein; for evidence of *Wolbachia* in sawflies, the only ancestral group for which previously no endosymbionts have been reported, see Graham *et al.*, 2008). We showed that haplodiploidy can be achieved in two different ways: either when the condition of high local relatedness is met or when the condition of direct endosymbiont benefits to their hosts is met. The first condition does not involve any assumption about the type of relationship the host has with its endosymbiont and both parasitic or mutualistic haploidizing bacteria could have induced haplodiploidy on their hosts. The second condition requires a strictly mutualistic relationship between the host and endosymbiont. Normark (2003) noted that four of 10 haplodiploid insect clades showed clear signs of inbreeding (regular brother–sister mating): the Hymenoptera, Thysanoptera and two bark beetle clades (Curculionidae: Scolytinae). According to our hypothesis, we would predict that haplodiploidy in those inbred groups is caused by endosymbionts that could either be parasitic or mutualistic. Nevertheless, if our hypothesis would work, haplodiploidy in the other six ancestral insect groups should all involve infection with endosymbionts that provide certain benefits to their hosts. A clear sign of such

mutualistic relationships are intricate host structures that interact and/or contain the endosymbionts, such as bacteriomes. Normark's analysis shows that such bacteriomes so far have only been observed in the noninbred clades (Normark, 2004b), which is roughly in line with our hypothesis that outbred haplodiploid ancestors always contain mutualistic bacteria. However, more information on the incidence of parasitic or mutualistic bacteria in haplodiploid ancestors is necessary to make a proper quantification of the importance of mutualistic host–endosymbiont relationships to the evolution of haplodiploidy.

The third condition for the evolution of EIH relates to the combination of high levels of endosymbiont transmission and high efficiencies of resource reallocation from killed males to infected females. These two conditions are important if haplodiploidy is to evolve via the condition of high local relatedness, but less so when the pathway to haplodiploidy is mediated by endosymbionts that are beneficial to the host. It is agreed that infection rates of male-killing endosymbionts appear to be generally high in nature (Hurst *et al.*, 2001; Jiggins *et al.*, 2002; Dyer & Jaenike, 2004; Charlat *et al.*, 2009), but the likelihood of high resource reallocation efficiencies has been debated (Engelstädter *et al.*, 2006; Ubeda & Normark, 2006). Fitness advantages for female offspring infected by male-killing endosymbionts due to reduced kin competition (Jaenike *et al.*, 2003) or cannibalism of killed males (Hurst *et al.*, 1993; Nakamura *et al.*, 2006) have been investigated in a number of organisms, but there is only a single study from which levels of b can be inferred (Dyer & Jaenike, 2004). As this study on male-killing endosymbionts in *Drosophila innubila* reports the fitness benefit of infected vs. uninfected females ($R \approx 1.04 - 1.05$), the survival rate of infected males ($s \approx 0 - 0.03$) and the transmission rate of the endosymbiont ($a \approx 0.97$), one can solve eqn 1 for b while assuming no direct fitness effects of the endosymbiont ($m = 0$). Inferred values of b are between 0.045 and 0.055. If such low levels of resource reallocation efficiencies are the norm in insects infected with male-killing bacteria, any increased levels of male viability despite infection would quickly reduce aR to levels equal or below 1, unless inbreeding is extremely high ($N < 1.023 - 1.028$ for the above values of b, see eqn S5). Given such low levels of resource reallocation, we can therefore expect that it is much more likely that endosymbionts achieve long-term persistence if they provide some additional benefits to their host.

Another assumption that only applies to our spatial model is female-biased dispersal. The combination of female-biased dispersal and inbreeding is observed in a number of haplodiploid groups, of which the two bark beetle clades Scolytinae and Xyleborini are the foremost examples. To a lesser extent, female-biased dispersal is also present in sawflies (Hymenoptera) and Thysanoptera, although the ancestral groups of the latter order are currently unresolved (Mound & Morris, 2007), making a characterization of ancestral traits difficult. To what extent inbreeding and female-biased dispersal have also played an important role in other haplodiploid groups is currently difficult to assess, due to the lack of well-resolved phylogenies, comparative data on the amount of inbreeding and information on dispersal asymmetries between the sexes. A systematic assessment of ancestral groups and their levels of inbreeding, dispersal asymmetries and prevalence of beneficial or parasitic endosymbionts may shed more light on the origins of haplodiploidy.

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Appendix A: Population dynamics in the nonspatial model

The number of uninfected (superscript 'u') and infected (superscript 'i') females (subscript 'f') and males (subscript

'm') can be described by the following recursion equations:

$$\begin{aligned} n_{\mathrm{f}}^{\mathrm{u}}(t+1) &= n_{\mathrm{f}}^{\mathrm{u}}(t) + n_{\mathrm{f}}^{\mathrm{i}}(t)(1-a)R \\ n_{\mathrm{f}}^{\mathrm{i}}(t+1) &= n_{\mathrm{f}}^{\mathrm{i}}(t)(1+m)\,aR \\ n_{\mathrm{m}}^{\mathrm{u}}(t+1) &= n_{\mathrm{f}}^{\mathrm{u}}(t) + n_{\mathrm{f}}^{\mathrm{i}}(t)(1-a)R \\ n_{\mathrm{m}}^{\mathrm{i}}(t+1) &= n_{\mathrm{f}}^{\mathrm{i}}(t)(1+m)\,aRs \end{aligned} \tag{3}$$

Uninfected mothers produce equal numbers of uninfected daughters and uninfected diploid sons. Infected mothers $n_{\mathrm{f}}^{\mathrm{i}}(t)$ obtain m additional resources compared with uninfected mothers due to direct benefits of possessing the endosymbiont. Furthermore, offspring from uninfected mothers receive R additional resources from their brothers who did not survive haploidization. Offspring of infected mothers are infected with probability a. If a son is infected, it will survive haploidization with probability s.

We can write the recursion equations above in matrix form $\mathbf{n}_{t+1} = \mathbf{A}\mathbf{n}_t$:

$$\begin{bmatrix} n_{\mathrm{f}}^{\mathrm{u}} \\ n_{\mathrm{f}}^{\mathrm{i}} \\ n_{\mathrm{m}}^{\mathrm{u}} \\ n_{\mathrm{m}}^{\mathrm{i}} \end{bmatrix}_{t+1} = \begin{bmatrix} 1 & (1-a)R & 0 & 0 \\ 0 & (1+m)\,aR & 0 & 0 \\ 1 & (1-a)R & 0 & 0 \\ 0 & (1+m)\,aRs & 0 & 0 \end{bmatrix} \begin{bmatrix} n_{\mathrm{f}}^{\mathrm{u}} \\ n_{\mathrm{f}}^{\mathrm{i}} \\ n_{\mathrm{m}}^{\mathrm{u}} \\ n_{\mathrm{m}}^{\mathrm{i}} \end{bmatrix}_{t}. \tag{4}$$

The transition matrix $\mathbf{A}$ has leading eigenvalues $\lambda_1 = 1$ and $\lambda_2 = (1 + m)aR$, with corresponding right eigenvectors $\mathbf{x}_1 = [1,0,1,0]$ and $\mathbf{x}_2 = [z,1,z,s]$, where $z = (1-a)R/(\lambda_2 - 1)$. Clearly, only if $\lambda_2 > \lambda_1$ can the endosymbiont persist, which results in inequality (2). In that case, the stable distribution of infected and uninfected females and males is given by the right eigenvector $\mathbf{x}_2$.

Appendix B: Mutant invasion dynamics in the nonspatial model

To investigate if a resident population with strategy $\{a^*,s^*\}$ is stable against the invasion of mutant strategies, recursion equations also need to include the contribution of males to the next generation, as mutant strategies can arise in either males or females. In that case, the state transition matrix $\mathbf{B}^*$ of the resident population is given by:

$$\mathbf{B}^* = \begin{bmatrix} \frac{1}{2} & \frac{1}{2}(1-a^*)R^* & \frac{1}{2}\left(p_1^*+p_2^*(1-a^*)R^*\right) & p_1^*+p_2^*(1-a^*)R^* \\ 0 & \frac{1}{2}(1+m)a^*R^* & \frac{1}{2}p_2^*(1+m)a^*R^* & p_2^*(1+m)a^*R^* \\ \frac{1}{2} & \frac{1}{2}(1-a^*)R^* & \frac{1}{2}\left(p_1^*+p_2^*(1-a^*)R^*\right) & p_1^*+p_2^*(1-a^*)R^* \\ 0 & \frac{1}{2}(1+m)a^*R^*s^* & 0 & 0 \end{bmatrix} \tag{5}$$

where $R^* = R(a^*,s^*)$ and $\{p_1,p_2\}$ are the expected numbers of mates per male with uninfected and infected females, respectively, in which x_j^k are the corresponding values from the leading right eigenvector $\mathbf{x}_2$:

$$p_1 = \frac{x_{\mathrm{f}}^{\mathrm{u}}}{x_{\mathrm{m}}^{\mathrm{u}} + x_{\mathrm{m}}^{\mathrm{i}}}, \quad p_2 = \frac{x_{\mathrm{f}}^{\mathrm{i}}}{x_{\mathrm{m}}^{\mathrm{u}} + x_{\mathrm{m}}^{\mathrm{i}}}.$$

The reproductive values of uninfected and infected females and males is given by the leading left eigenvector $\mathbf{v}$:

$$\mathbf{v} = [1, 2(1-a^*)R^* + 2s^*(2\lambda_2 - 1), 2\lambda_2 - 1, 4\lambda_2 - 2]. \tag{6}$$

In the rest of this appendix, we will use these reproductive values to derive selection gradients for s and a for four different combinations of host and endosymbiont control.

B.1. Host control of *a*

The invasion prospects of a rare mutant with strategy a in a population of residents with strategy a^* is governed by the transition matrix $\mathbf{B}$. Following Taylor (1996), the fitness gradient for the mutant strategy a can be calculated as follows:

$$\left.\frac{\partial W}{\partial a}\right|_{a=a^*} = \sum_{i,j} v_i x_j \left.\frac{\partial b_{ij}}{\partial a}\right|_{a=a^*} \tag{7}$$

which involves only taking into account the elements of matrix $\mathbf{B}$ (second column) that are dependent on the mutant strategy a:

$$\begin{aligned} \left.\frac{\partial W}{\partial a}\right|_{a=a^*} &= (v_{\mathrm{f}}^{\mathrm{u}} + v_{\mathrm{m}}^{\mathrm{u}})(-R^* + (1-a^*)R_a^*) \\ &\quad + (v_{\mathrm{f}}^{\mathrm{i}} + v_{\mathrm{m}}^{\mathrm{i}}s)(R^* + a^*R_a^*)(1+m) \end{aligned} \tag{8}$$

where R_a^* is $\partial R/\partial a|_{a=a^*}$.

B.2. Host control of *s*

If the maternal host is in control of haploidized male viability, the invasion prospects of a rare mutant with strategy s is governed by the transition matrix $\mathbf{B}$, where R in the second column is replaced by:

$$R = 1 + \frac{\frac{1}{2}ba^*((1-s) + (1-s^*))}{2 - \frac{1}{2}a^*((1-s) + (1-s^*))} \tag{9}$$

as half of the haploidized male offspring produced by a heterozygous mutant mother will carry the mutant allele. The fitness gradient then becomes:

$$\begin{aligned} \left.\frac{\partial W}{\partial s}\right|_{s=s^*} &= \left(v_{\mathrm{f}}^{\mathrm{u}} + v_{\mathrm{m}}^{\mathrm{u}}\right)(1-a^*)R_s^* + \left(v_{\mathrm{f}}^{\mathrm{i}} + v_{\mathrm{m}}^{\mathrm{i}}s^*\right)(1+m)a^*R_s^* \\ &\quad + v_{\mathrm{m}}^{\mathrm{i}}(1+m)a^*R^* \end{aligned} \tag{10}$$

B.3. Endosymbiont control of *a*

As from the viewpoint of a haploidizing endosymbiont, uninfected daughters and sons have zero reproductive

value, selection will maximize the number of infected daughters produced by infected mothers, given by $(1 + m)aR$. The selection gradient is therefore given by:

$$\left.\frac{\partial W}{\partial a}\right|_{a=a^*} = (1+m)R^* + a^*(1+m)R_a^*. \qquad (11)$$

where $R_a = \partial R/\partial a|_{a=a^*} > 0$. The selection gradient is always positive, hence the endosymbiont always favours increasing its own transmission rate.

B.4. Endosymbiont control of *s*

By the same reasoning as in Appendix B.3, selection on endosymbiont control of s maximizes $(1 + m)aR$; hence, the selection gradient is given by:

$$\left.\frac{\partial W}{\partial s}\right|_{s=s^*} = a^*(1+m)R_s^* \qquad (12)$$

where $R_s^* = \partial R/\partial s|_{s=s^*} < 0$. Thus, selection favours zero survival of haploidized males.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Extreme inbreeding facilitates haplodiploidy.

Figure S1 Ten replicate simulations showing the rise and fall of the male haploidizing endosymbiont and the transient presence of haploidized males in the population.

Figure S2 Ten replicate simulations showing the rise and subsequent decline of the endosymbiont, when it is in control of its own transmission rate, whereas the host controls s.

Figure S3 Stable persistence of haplodiploidy is only possible under very high local relatedness.

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