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EVOLUTION OF INCOMPATIBILITY-INDUCING MICROBES IN SUBDIVIDED HOST POPULATIONS

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Many insects, other arthropods, and nematodes harbor maternally inherited bacteria inducing "cytoplasmic incompatibility" (CI), reduced egg hatch when infected males mate with uninfected females. Although CI drives the spread of these microbes, selection on alternative, mutually compatible strains in panmictic host populations does not act directly on CI intensity but favors higher "effective fecundity," the number of infected progeny an infected female produces. We analyze the consequences of host population subdivision using deterministic and stochastic models. In subdivided populations, effective fecundity remains the primary target of selection. For strains of equal effective fecundity, if population density is regulated locally (i.e., "soft selection"), variation among patches in infection frequencies may induce change in the relative frequencies of the strains. However, whether this change favors stronger incompatibility depends on initial frequencies. Demographic fluctuations maintain frequency variation that tends to favor stronger incompatibility. However, this effect is weak; even with small patches, minute increases in effective fecundity can offset substantial decreases in CI intensity. These results are insensitive to many details of host life cycle and migration and to systematic outbreeding or inbreeding within patches. Selection acting through transfer between host species may be required to explain the prevalence of CI.

KEY WORDS: **Clade selection, kin selection, modifier evolution, spite, symbiont evolution, *Wolbachia*.**

Many insects, other arthropods, and nematodes are infected with *Wolbachia* or *Cardinium*, maternally inherited bacteria inducing reproductive incompatibility between infected males and uninfected females (Hoffmann and Turelli 1997; Werren 1997; Weeks et al. 2002; Zchori-Fein and Perlman 2004). In diploid hosts, incompatible crosses yield fewer adult offspring due to reduced egg hatch. Because the bacteria are cytoplasmically transmitted, the incompatibility they induce is known as cytoplasmic incompatibility (CI). In many hosts, mortality of uninfected embryos outweighs other effects of the bacteria on host fitness (Hoffmann and Turelli 1997), and host mating is effectively random with respect to infection status (Hoffmann et al. 1990; Champion de Crespigny and Wedell 2007). Accordingly, CI-causing bacteria tend to spread within host species, because in populations polymorphic for infection, infected females, whose infected eggs are compatible with sperms from both infected and uninfected males, tend to produce more progeny than uninfected females (Caspari and Watson 1959). This propensity is qualified by imperfect transmission—infected females may lay some uninfected eggs, which are vulnerable to CI—and fecundity reduction—infected females may lay fewer eggs. Both phenomena are common in nature and lead to a threshold infection frequency, which the bacteria must surpass by chance or migration to attain a nonzero stable equilibrium

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infection frequency (Caspari and Watson 1959; Fine 1978; Hoffmann et al. 1990; Jansen et al. 2008).

Because CI drives their spread, it might be supposed that selection on CI-causing bacteria within a given host species favors stronger CI (i.e., fewer adult offspring from incompatible crosses). On the contrary, Prout (1994) and Turelli (1994) showed that in panmictic host populations, selection on alternative, mutually compatible bacterial strains does not favor stronger CI. Instead, the target of selection is the number of infected eggs an infected female lays (i.e., the product of her fecundity and transmission efficiency), which we refer to as the effective fecundity of the strain infecting her. The strain of higher effective fecundity displaces the other, regardless of its CI intensity (Turelli 1994). This conclusion is consistent with recent data regarding *Wolbachia* evolution. The *Wolbachia* infection in California populations of *Drosophila simulans* typifies the rapid spread of CI-causing bacteria in nature (Turelli and Hoffmann 1991, 1995). As predicted by Prout and Turelli, who ignored the possibilities of host population structure and bacterial variation within host individuals, the *Wolbachia* in California *D. simulans* have evolved over the past 15 years so that effective fecundity has risen (Weeks et al. 2007); in the early 1990s, infected females usually laid fewer eggs than uninfected females in the laboratory (Hoffmann et al. 1990; Nigro and Prout 1990), whereas now, infected females often lay more eggs. (This evolution has not occurred in laboratory stocks, so *Wolbachia* with the earlier, parasitic phenotype or the later, mutualistic phenotype can be compared contemporaneously.) In contrast, CI intensity has not changed appreciably (as measured using reference, uninfected laboratory strains), and the parasitic and mutualistic *Wolbachia* strains are mutually compatible (Weeks et al. 2007).

CI has been described in terms of two separate functions (Hurst 1991; Hurst and McVean 1996): one, termed "mod" by Werren (1997), modifies sperms so that they are incompatible with uninfected eggs; the other, termed "resc," rescues infected eggs from the embryonic mortality otherwise inflicted by modified sperms. Within a given host species, there can be multiple bacterial strains that are mutually incompatible, as first described by Laven (1959) in *Culex pipiens* and subsequently described in other hosts (e.g., O'Neill and Karr 1990; Montchamp-Moreau et al. 1991). Several recent studies treat the evolution of new compatibility types (e.g., Charlat et al. 2001; Engelstädter et al. 2006). We consider the simpler case of bacterial strains that are mutually compatible, meaning each can rescue eggs from sperms modified by the others; in Werren's (1997) terminology, we assume that all strains are $resc^+$ (i.e., infected eggs are compatible with infected sperms) but may be either mod^- or mod^+ (i.e., infected sperms may be either compatible or incompatible with uninfected eggs). Comparative data indicate that mutually incompatible *Wolbachia* strains do not arise often or quickly, even when bacteria are transferred between hosts (Bourtzis et al. 1998; Charlat et al. 2002; Zabalou et al. 2008). In contrast, several pairs of closely related and mutually compatible *Wolbachia* strains are known, where one causes CI (mod^+resc^+) and the other does not (mod^-resc^+) (Bourtzis et al. 1998; Merçot and Poinsot 1998). The analyses of Prout (1994) and Turelli (1994) predicted that a mod^-resc^+ mutant of a mod^+resc^+ strain would spread if it raised effective fecundity. Thus, both theory and data suggest that bacterial evolution within a given host species does not tend to strengthen CI.

However, the analyses of Prout (1994) and Turelli (1994) ignored host population subdivision, which is clearly pervasive in nature (Coyne et al. 2000). Moreover, it has been argued that host population subdivision engenders selection for stronger CI (Hurst 1991; Frank 1997). Therefore, in this article, we analyze the consequences of host population subdivision for the evolution of alternative, mutually compatible strains of CI-causing bacteria within a given host species.

The reason CI intensity is a neutral trait in panmictic host populations is that alternative, mutually compatible bacterial strains benefit equally from the additional mortality of uninfected embryos when one strain causes stronger CI than the other. For selection to favor stronger CI, the infected progeny of females carrying a stronger strain must benefit preferentially from the additional mortality inflicted by males carrying the strain. Several scenarios have been discussed or analyzed that might fulfill this requirement. Hurst (1991) proposed that CI could evolve through a kin-selection mechanism analogous to Hamilton's (1970) proposal for the evolution of "spiteful" behavior (i.e., behavior that harms the actor but harms the recipient more). Specifically, he argued that with sib-mating and local density regulation, males carrying a stronger strain could preferentially benefit the infected progeny of their sisters, through reduced larval competition. The crucial features of this verbal model are population subdivision and local regulation. It is notable that in a subsequent study, Hurst and McVean (1996), building on the analyses of Prout (1994) and Turelli (1994), argued for an evolutionary trend toward weaker CI within a given host species. After implicitly rejecting Hurst's (1991) kin-selection mechanism as a general explanation for the prevalence of CI, they invoked clade selection for producing CI in novel hosts as a plausible alternative, an idea that had previously been advocated by Hurst et al. (1992). We return to this idea under "Discussion" below. Ambiguity persists in the literature about conditions favoring the spread of CI-causing bacteria per se and those favoring the spread of stronger versus weaker strains (e.g., Rousset and Raymond 1991; Gardner and West 2004; Engelstädter and Charlat 2006). We focus on the latter.

Hurst's (1991) kin-selection mechanism was elaborated by Frank (1997) in a model featuring host population subdivision with local density regulation and emphasizing "the kin selection coefficient of relatedness" within host groups. The coefficient r

is defined as "the slope of group phenotype [y] on individual genotype [x]," where x is the CI intensity of a random infected female and y is the average CI intensity of the infected males in her group. Frank treated r as a free parameter, and his analysis suggested that selection favors stronger CI whenever r is positive. However, Frank's treatment lacked an explicit model of population structure or an explicit analysis of the frequency dynamics of alternative strains, leaving the generality of its conclusions and the strength of the selection on CI intensity unclear. Given the ubiquity of host population subdivision, clarifying its implications for the evolution of CI-causing bacteria is important.

We analyze the evolution of CI-causing bacteria, supposing that alternative strains differ in intensity of incompatibility between infected males and uninfected females and perhaps in fecundity and/or transmission efficiency of infected females. We suppose that the strains are completely compatible with each other (cf. Turelli 1994 and Engelstädter et al. 2006 for analyses of incompatible strains) and ignore the possibility of bacterial variation within host individuals. We refer to change in the relative frequencies of the strains as CI evolution. Of particular interest is whether host population subdivision engenders selection for stronger CI, as argued by Hurst (1991) and Frank (1997). We suppose that hosts occupy patches connected by migration. This is reasonable, in that many hosts of CI-causing bacteria spend most of their lives in discrete patches of suitable habitat (e.g., Werren 1983; Hoffmann and Nielsen 1985). For simplicity, we suppose that host generations do not overlap. Only numerical analyses of CI with overlapping generations have appeared (Rasgon et al. 2003; Rasgon and Scott 2004), but both numerical and algebraic analyses (M. Turelli, unpubl. ms.) yield results qualitatively similar to those with nonoverlapping generations. We suppose that hosts are diploid but hermaphroditic, to avoid the algebraic complications of separate sexes. We also omit paternal and horizontal transmission, which are rare in nature (Hoffmann et al. 1990; Turelli et al. 1992; Turelli and Hoffmann 1995; Vavre et al. 1999). Because the mechanisms of CI are not understood yet, we assume no particular relationships among the parameters governing its dynamics; our goal is to understand the dynamics of strains that may differ in CI intensity, fecundity, and/or transmission efficiency independently. After showing that the dynamics are governed primarily by effective fecundity, we assume equal effective fecundity to explore the secondary effect of differences in CI intensity.

We first review CI evolution in a panmictic host population, considering both a deterministic model and stochastic effects in a finite population. This introduces our notation and provides the background needed for understanding the consequences of population subdivision. Our deterministic model slightly extends that of Turelli (1994) by letting infection raise rather than lower female fecundity, as observed in several *Wolbachia*–host associations (Dobson et al. 2002; Weeks et al. 2007). We also consider systematic outbreeding and inbreeding, which figure prominently in recent treatments of "spiteful" cytoplasmic elements (Engelstädter and Charlat 2006) and the evolution of new compatibility types (Engelstädter et al. 2006). We then analyze CI evolution in a subdivided host population, again considering both deterministic models and stochastic effects and systematic outbreeding and inbreeding. For readers uninterested in formal derivations, we summarize our biological conclusions at the outset of each section or subsection.

Models and Analyses

PANMICTIC POPULATIONS

We first introduce parameters of bacterial strains and variables of host populations. Table 1 is a glossary of notation for the entire article. We then analyze a deterministic model of CI evolution

Table 1. Glossary of notation.

Symbol	Definition
f	fraction of hosts that are female
F_i	fecundity of an I_i female relative to a U female
φ_i	$F_i(1-\mu_i)$, effective fecundity of an I_i female relative to a U female
H_i	hatch rate from U♀ × I_i♂ relative to _♀ × U♂
I_i	parasite strain i (i=1, 2)
K	number of hosts in patch
L	number of larvae from U♀ × U♂
m	for stepping-stone connectivity, fraction of hosts exchanged with adjacent patches; for migrant-pool connectivity, fraction of hosts exchanged with pool
μ_i	fraction of U eggs an I_i female lays
N	number of patches
$^{(n)}$	(superscript) in patch n (n=1, 2, . . . N)
P	overall number of infected hosts ($=P_1+P_2$)
p	overall frequency of infected hosts ($=p_1+p_2$)
$\hat{p}(R_1)$	stable deterministic equilibrium value of p, for given R_1
$\check{p}(R_1)$	unstable deterministic equilibrium value of p, for given R_1
P_i	number of I_i hosts ($=PR_i$)
p_i	frequency of I_i hosts ($=pR_i$)
Q	number of U hosts ($=1-P$)
q	frequency of U hosts ($=1-p$)
R_i	relative frequency of I_i hosts ($=p_i/p$)
s_{f_i}	$1-F_i$, the fecundity effect of I_i
s_{h_i}	$1-H_i$, the CI intensity of I_i
U	uninfected
v	fraction of larvae that survive to maturity
$'$	(superscript) after migration
$''$	(superscript) after hatching
$'''$	(superscript) after maturation
_	any infection status

in a panmictic population, recapitulating and slightly extending Turelli (1994). The main result is that the target of selection is effective fecundity. We then discuss the consequences of sib-mating avoidance or preferential sib-mating, which accelerate or decelerate, respectively, the spread of all strains but do not promote stronger or weaker CI. Finally, we consider stochastic effects in a finite panmictic population and conclude that genetic drift does not systematically affect CI intensity.

Our notation follows that of Turelli (1994). Let I_1 and I_2 denote bacterial strains. We suppose that I_1 and I_2 are completely compatible with each other and that no host is infected with I_1 and I_2 simultaneously. We refer to hosts infected with I_i as I_i hosts and to uninfected hosts as U hosts. Let H_i denote the hatch rate from an incompatible cross, U♀ × I_i♂, relative to a compatible cross, _♀ × U♂, where _ denotes any infection status, and let $s_{h_i} = 1 - H_i$ denote the CI intensity of I_i; $H_i \geq 0$, hence $s_{h_i} \leq 1$, and we suppose that $H_i \leq 1$, hence $s_{h_i} \geq 0$. Let μ_i denote the transmission inefficiency of an I_i female (i.e., the fraction of U eggs an I_i female lays); $0 \leq \mu_i \leq 1$. (An alternative form of imperfect transmission, which would alter the formalism only slightly, is that some infected progeny are "cured" by exposure to naturally occurring antibiotics; cf. Stevens and Wicklow 1992; Clancy and Hoffmann 1998.) Let F_i denote the fecundity of an I_i female relative to a U female, and let $s_{f_i} = 1 - F_i$ denote the fecundity effect of I_i; $F_i \geq 0$, hence $s_{f_i} \leq 1$. s_{f_i} can be negative to accommodate bacteria that raise fecundity, but we will not consider the extreme case of bacteria that are required for fecundity (Hoerauf et al. 1999; Dedeine et al. 2004; Pannebakker et al. 2007).

In nature, hatch rates, transmission inefficiencies, and fecundities vary, even among hosts with a given infection status. We neglect this, as do most treatments of CI dynamics (but cf. Guillemaud and Rousset 1997). H_i, μ_i, and F_i may be regarded as mean values. This neglects variances and covariances of hatch rates, transmission inefficiencies, and fecundities. The variances are certainly nonzero (Turelli and Hoffmann 1995), and the covariances may be nonzero (Boyle et al. 1993; Breeuwer and Werren 1993; Turelli and Hoffmann 1995). However, neglecting them is defensible, as simple modeling with mean values yields good agreement with many observations of CI dynamics (Turelli and Hoffmann 1995), and more complex modeling is less likely to yield insights by merely positing variances and covariances than by explicitly representing mechanisms that generate them. It is useful to have in mind some representative parameter values. For *Wolbachia* in California *D. simulans*, Turelli and Hoffmann (1995) estimated $H \approx 0.55$, $\mu \approx 0.04$, and $F \approx 1$, although more recent data suggest $F > 1$ (Weeks et al. 2007); in California *C. pipiens*, Rasgon and Scott (2003) estimated $H \approx 0$, $\mu \approx 0.01$, and $F \approx 1$; and in Australian *D. melanogaster*, Hoffmann et al. (1994, 1998) estimated $H \approx 0.15$, $\mu \approx 0.025$, and $F \approx 1$. Thus, a wide range of CI intensities have been observed in nature, and transmission is typically somewhat imperfect.

Table 2. Basis of equations (1) and (2).

Mating		(Offspring per mating)/L		
♀×♂	frequency	I_1	I_2	U
I_1×I_1	p_1^2	$F_1(1-\mu_1)$		$F_1\mu_1H_1$
I_1×I_2	p_1p_2	$F_1(1-\mu_1)$		$F_1\mu_1H_2$
I_1×U	p_1q	$F_1(1-\mu_1)$		$F_1\mu_1$
I_2×I_1	p_2p_1		$F_2(1-\mu_2)$	$F_2\mu_2H_1$
I_2×I_2	p_2^2		$F_2(1-\mu_2)$	$F_2\mu_2H_2$
I_2×U	p_2q		$F_2(1-\mu_2)$	$F_2\mu_2$
U×I_1	qp_1			H_1
U×I_2	qp_2			H_2
U×U	q^2			1

Deterministic analysis with random mating

Let p_i denote the frequency of adult I_i hosts and q the frequency of adult U hosts, so $q = 1 - p_1 - p_2$. Assuming that uninfected eggs of infected females are incompatible with sperms of infected males (Turelli and Hoffmann 1995), Table 2 implies that the change in p_i over one generation is

$$\Delta p_i = \frac{p_i[F_i(1-\mu_i) - \overline{W}]}{\overline{W}}, \text{ where} \tag{1a}$$

$$\overline{W} = p_1F_1(1-\mu_1) + p_2F_2(1-\mu_2) + (p_1F_1\mu_1 + p_2F_2\mu_2 + q)(p_1H_1 + p_2H_2 + q). \tag{1b}$$

Let p denote the overall frequency of infection among adults, so $p = p_1 + p_2$, and let R_i denote the relative frequency of I_i among infected adults,

$$R_i = \frac{p_i}{p}, \tag{2}$$

so $R_2 = 1 - R_1$. In terms of p and R_1, (1) is equivalent to

$$\Delta p = \frac{p[R_1F_1(1-\mu_1) + R_2F_2(1-\mu_2) - \overline{W}]}{\overline{W}}, \text{ where} \tag{3a}$$

$$\overline{W} = p[R_1F_1(1-\mu_1) + R_2F_2(1-\mu_2)] + [p(R_1F_1\mu_1 + R_2F_2\mu_2) + q] \times [p(R_1H_1 + R_2H_2) + q], \tag{3b}$$

and

$$\Delta R_1 = \frac{R_1R_2[F_1(1-\mu_1) - F_2(1-\mu_2)]}{R_1F_1(1-\mu_1) + R_2F_2(1-\mu_2)}. \tag{4}$$

The sign of ΔR_1 is the sign of $F_1(1-\mu_1) - F_2(1-\mu_2)$, where $F_i(1-\mu_i)$ is the effective fecundity of I_i females relative to U females, that is, the number of infected eggs laid by an I_i female relative to the number of eggs laid by a U female. Thus, as shown by Turelli (1994), the target of selection in a panmictic population is effective fecundity.

It will prove useful to consider the dynamics and their equilibria when the strains have equal effective fecundity. If $F_1(1-\mu_1) = F_2(1-\mu_2)$, (3) and (4) become

$$\Delta p = \frac{p[F_1(1-\mu_1) - \overline{W}]}{\overline{W}}, \text{ where} \tag{5a}$$

$$\overline{W} = pF_1(1-\mu_1) + [p(R_1F_1\mu_1 + R_2F_2\mu_2) + q] \times [p(R_1H_1 + R_2H_2) + q], \text{ and} \tag{5b}$$

$$\Delta R_1 = 0. \tag{6}$$

For each value of R_1, there is at most one nonzero stable equilibrium value of p. Let $\overline{s_h} = R_1 s_{h_1} + R_2 s_{h_2}$ and $\overline{s_f} = R_1 s_{f_1} + R_2 s_{f_2}$ denote the average CI intensity and fecundity effect, respectively. Assuming for simplicity that $\overline{s_h} + \overline{s_f} > 0$, let

$$\hat{p}(R_1) = \frac{\overline{s_h} + \overline{s_f} + \sqrt{(\overline{s_h} + \overline{s_f})^2 - 4\overline{s_h}(1-\overline{F\mu})(\overline{s_f} + \overline{F\mu})}}{2\overline{s_h}(1-\overline{F\mu})}, \tag{7a}$$

where

$$\overline{F\mu} = R_1F_1\mu_1 + R_2F_2\mu_2. \tag{7b}$$

A straightforward analysis of (5) shows that $\hat{p}(R_1)$ is an equilibrium if it is feasible (i.e., $0 \le \hat{p}(R_1) \le 1$), and it is stable if the argument of the square root in (7a) is positive. When $\hat{p}(R_1)$ is stable, there is also an unstable equilibrium $\check{p}(R_1)$. Assuming for simplicity that $F_1(1-\mu_1) < 1$, $\check{p}(R_1)$ is between 0 and $\hat{p}(R_1)$ and is obtained by changing the sign of the square root in (7a). Assuming that $\hat{p}(R_1)$is stable, $(\hat{p}(R_1),\ R_1)$ is an equilibrium of (5) and (6), stable with respect to perturbations in p and neutral with respect to perturbations in R_1. Generically, a trajectory approaches either $(\hat{p}(R_1),\ R_1)$ (i.e., both strains persist, and their relative frequencies do not change) or $(0, R_1)$ (i.e., neither strain persists), depending on whether the initial value of p is greater or less than $\check{p}(R_1)$.

Deterministic analysis with systematic outbreeding or inbreeding

Engelstädter and Charlat (2006) analyzed the population dynamics of "spiteful cytoplasmic elements" functionally similar to CI-causing bacteria but with infected females incompatible with infected males. They argued that such elements tend to spread within host species with sib-mating avoidance. This is intuitively evident, in that sib-mating avoidance raises the frequency of incompatible matings an uninfected female experiences and lowers the frequency of incompatible matings an infected female experiences. This argument resembles those made for kin selection leading to stronger CI, so it is worth understanding how sib-mating avoidance or preferential sib-mating affects the evolution of CI-causing bacteria. Engelstädter et al. (2006) elaborated Engelstädter and Charlat's (2006) model to simulate the evolution of new compatibility types, but they did not investigate the effects of nonrandom mating on the evolution of CI intensity. These are easy to see assuming perfect maternal transmission of each bacterial strain, but imperfect transmission does not substantively alter the outcome.

Assuming perfect transmission, the number of progeny an I_i female produces is proportional to F_i, regardless of the infection status of her mate, so it makes no difference how many brothers she has with whom she avoids or prefers mating. Suppose that an uninfected female has k brothers among N_m potential mates. By avoiding her brothers, she changes her expected number of progeny from

$$w_U = q + p_1H_1 + p_2H_2 \tag{8a}$$

to

$$\tilde{w}_U = \frac{qN_m - k}{N_m - k} + \frac{p_1N_m}{N_m - k}H_1 + \frac{p_2N_m}{N_m - k}H_2. \tag{8b}$$

Assuming that $H_1 \le 1$ and $H_2 \le 1$, $\tilde{w}_U < w_U$ whenever $q < 1$, and hence, the infections spread more quickly with sib-mating avoidance. By similar reasoning, the infections spread more slowly with preferential sib-mating. However, the relative fitnesses of the alternative infections are not affected. Thus, neither sib-mating avoidance nor preferential sib-mating promotes stronger or weaker CI in a panmictic population.

Stochastic effects

So far, our analyses are deterministic. However, two classes of stochastic factors are biologically pertinent. One comprises individual-level factors such as variation in fecundity among females with a given infection status. As explained above, we neglect such factors. The other class comprises population-level factors such as sampling deviations of infection frequencies among mature hosts from those among larvae. Qualitatively, if the population is near a nonzero stable deterministic equilibrium, such factors induce fluctuations about it. Eventually, the population will fluctuate below the threshold infection frequency, whereupon loss of infection is likely. However, this may take a very long time. Meanwhile, if $F_1(1-\mu_1) = F_2(1-\mu_2)$, genetic drift in R_1 is

unopposed. Eventually, one strain will displace the other by chance. On the face of it, there is no reason to suspect that genetic drift in R_1 is biased with respect to CI intensity, and a straightforward analysis confirms this impression. This is noteworthy because, as shown below, in subdivided populations, genetic drift in R_1 is biased toward stronger CI. (However, as pointed out by a reviewer, it is plausible that mutation preferentially weakens rather than strengthens CI, in which case the combination of mutation and drift would tend to weaken CI in panmictic populations and perhaps even in subdivided ones.)

SUBDIVIDED POPULATIONS

In this section, we analyze CI evolution in subdivided populations, considering first deterministic models and then stochastic effects. We suppose that hosts occupy patches connected by migration, with local density regulation. We also consider the consequences of systematic outbreeding and inbreeding within patches. The questions we address are how population subdivision, with variation among patches in both the overall frequency of infection and the relative frequencies of the alternative strains, influences CI evolution and how sib-mating avoidance or preferential sib-mating within patches modifies this.

With population subdivision, the new element of the life cycle is migration. This can occur at several stages, and its quantitative effects depend on its placement. We consider two possibilities: (1) migration before mating—from each patch, a certain proportion of mature hosts emigrate, and subsequently, in each patch, both residents and immigrants mate randomly; or (2) migration after mating—in each patch, mature hosts mate either randomly or nonrandomly, and subsequently, from each patch, a certain proportion of mated females emigrate. Both life cycles are common in nature. Migration before mating is simpler to analyze. There are many plausible migration schemes, corresponding to various distributions of patches in space and various degrees of long-distance versus local dispersal. Again, we consider two possibilities, representing different degrees of long-distance versus local dispersal: (1) stepping-stone connectivity—the patches form a ring, and each patch exchanges migrants with its nearest neighbors only; or (2) migrant-pool connectivity—each patch exchanges migrants with all the others through a migrant pool. For simplicity, our algebraic treatment focuses on migration before mating and stepping-stone connectivity, but Appendix 1 gives some details of the other three cases, and our numerical examples encompass all four cases.

We label variables of patch n with the superscript $^{(n)}$, where n varies from 1 to N, the number of patches; for example, $p^{(n)}$ denotes the overall frequency of infection in patch n. Otherwise, we retain the notation of the preceding section; for example, R_1 denotes the relative frequency of I_1 in the entire population.

Deterministic analysis with random mating

In this subsection, we show that in a subdivided population, the primary target of selection is effective fecundity, much as in a panmictic population. For strains of equal effective fecundity, if larval density is regulated locally (i.e., soft selection), variation among patches may induce CI evolution, but whether this favors stronger incompatibility depends on the initial condition of the population. Moreover, the effect of subdivision is transient, because migration homogenizes the patches.

We focus on ΔR_1, the change in the relative frequency of I_1 in the entire population over one generation. The derivation of a formula for ΔR_1 is more transparent in terms of numbers of hosts than in terms of frequencies of strains alone. However, despite referring to numbers of hosts, we neglect sampling deviations, although they are inevitable in finite populations. We return to this issue under "Stochastic effects" below.

Let K denote the number of mature hosts in each patch before migration, and for stepping-stone connectivity, suppose that a fraction m of hosts emigrate from each patch to the nearest neighboring patches. (Patch-dependent carrying capacities and migration rates complicate the notation but have no general, qualitative significance.) Let

$$\ell(n) = n - 1 \quad \text{if } n > 1 \quad \text{and} \quad \ell(1) = N, \tag{9a}$$

$$r(n) = n + 1 \quad \text{if } n < N \quad \text{and} \quad r(N) = 1 \tag{9b}$$

(i.e., the patches are numbered around a circle). Then the number of I_i hosts in patch n after migration is

$$P_i^{(n)\prime} = p_i^{(n)} K(1-m) + Km\left(p_i^{(\ell(n))} + p_i^{(r(n))}\right)/2. \tag{10}$$

Suppose that a fraction f of hosts in each patch is female, and an infection-free cross U♀ × U♂ yields L larvae. Then reasoning as in Table 2, the number of I_i hosts in patch n after hatching is

$$P_i^{(n)\prime\prime} = P_i^{(n)\prime} f L F_i (1-\mu_i). \tag{11}$$

Finally, suppose that a (density-dependent) fraction $v^{(n)}$of larvae in patch n survives to maturity (we consider the details of the density dependence below). Then the number of I_i hosts in patch n after a generation is

$$P_i^{(n)\prime\prime\prime} = P_i^{(n)\prime\prime} v^{(n)}. \tag{12}$$

Migration merely redistributes the population, so it does not change R_1 (i.e., $R_1^{'} = R_1$); this is intuitively obvious and easily confirmed using (10). Hence by (12), (11), and algebra,

$$\Delta R_1 = \frac{\sum_n P_1^{(n)'''}}{\sum_n \left(P_1^{(n)'''} + P_2^{(n)'''}\right)} - \frac{\sum_n P_1^{(n)'}}{\sum_n \left(P_1^{(n)'} + P_2^{(n)'}\right)} \tag{13a}$$

$$= \frac{[F_1(1-\mu_1) - F_2(1-\mu_2)]\left(\sum_n P_1^{(n)'} v^{(n)}\right)\left(\sum_n P_2^{(n)'}\right)}{D}$$

$$+ \frac{F_2(1-\mu_2)\sum_{m<n}\left(P_1^{(m)'}P_2^{(n)'} - P_1^{(n)'}P_2^{(m)'}\right)\left(v^{(m)} - v^{(n)}\right)}{D}, \tag{13b}$$

where the common denominator is

$$D = \left(\sum_n \left[P_1^{(n)'}F_1(1-\mu_1) + P_2^{(n)'}F_2(1-\mu_2)\right] v^{(n)}\right) \times \left(\sum_n \left(P_1^{(n)'} + P_2^{(n)'}\right)\right), \tag{13c}$$

which is positive. The sign of the first term in (13b) is the sign of $F_1(1-\mu_1) - F_2(1-\mu_2)$, so this term favors the strain of higher effective fecundity. The second term in (13b) is nonzero only if infection frequencies after migration and larval viability vary among patches, so this term approaches zero as migration homogenizes the patches. For strains of different effective fecundity, the first term will be decisive.

To understand the effects of patch heterogeneity, assume equal effective fecundity. If $F_1(1-\mu_1) = F_2(1-\mu_2)$, the sign of ΔR_1 is the sign of the sum in the numerator of the second term in (13b), which may be rewritten

$$\sum_{m<n} P^{(m)'}P^{(n)'}\left(R_1^{(m)'} - R_1^{(n)'}\right)\left(v^{(m)} - v^{(n)}\right), \tag{14}$$

where $P^{(n)'} = P_1^{(n)'} + P_2^{(n)'}$. This contains a term per pair of patches, which is positive if the relative frequency of both I_1 after migration and larval viability is greater in the same one of the two patches. Thus, I_1 is favored over I_2 when relative abundance of I_1 after migration is positively correlated with larval survival to maturity. Whether such a correlation exists depends on how larval viability is determined.

A plausible supposition is that density-dependent factors such as competition among larvae for resources set the number of mature hosts in each patch before migration to K each generation, regardless of infection frequencies among the larvae. This constitutes local density regulation (also known as soft selection; Christiansen 1975). Assuming that all larvae are equally competitive,

$$v^{(n)} = \frac{K}{P_1^{(n)''} + P_2^{(n)''} + Q^{(n)''}}, \tag{15}$$

where $Q^{(n)''}$ is the number of U hosts in patch n after hatching. For migration before mating,

$$Q^{(n)''} = \left[p^{(n)'}\left(R_1^{(n)'}F_1\mu_1 + R_2^{(n)'}F_2\mu_2\right) + q^{(n)'}\right] \times \left[p^{(n)'}\left(1 - \left(R_1^{(n)'}s_{h_1} + R_2^{(n)'}s_{h_2}\right)\right) + q^{(n)'}\right] K' f L, \tag{16}$$

where K' is the number of mature hosts in each patch after migration; $K' = K$ if migration is uniform across patches and free of mortality. The first term in brackets is proportional to the number of uninfected eggs in the patch and the second to their average hatch rate.

To understand when (14) is positive, we must understand how $v^{(n)}$ varies with $R_1^{(n)'}$. If $F_1(1-\mu_1) = F_2(1-\mu_2)$,

$$\frac{\partial v^{(n)}}{\partial R_1^{(n)'}} = A_h\left(s_{h_1} - s_{h_2}\right) + A_f(F_2\mu_2 - F_1\mu_1), \tag{17}$$

where A_h and A_f are nonnegative quantities given in Appendix 2. If I_1 has greater CI intensity ($s_{h_1} > s_{h_2}$) and/or I_1 females lay fewer U eggs ($F_1\mu_1 < F_2\mu_2$), larval survival to maturity increases with relative abundance of I_1 after migration. For such I_1, greater relative abundance of I_1 after migration entails fewer larvae—uninfected females produce fewer progeny, because CI intensity among their mates is greater, and infected females produce fewer uninfected progeny, because CI intensity among their mates is greater and/or because they lay fewer uninfected eggs. And with local density regulation, fewer larvae entail greater larval survival to maturity. This is central to the kin-selection scenarios of Hurst (1991) and Frank (1997). However, even if $s_{h_1} > s_{h_2}$, $F_1\mu_1 < F_2\mu_2$, and $R_1^{(m)'} > R_1^{(n)'}$, $v^{(m)} < v^{(n)}$ is possible, because $v^{(n)}$depends on not only $R_1^{(n)'}$ but also $p^{(n)'}$.

Complementary to (17),

$$\frac{\partial v^{(n)}}{\partial p^{(n)'}} = A_p\left(\frac{\hat{p}\left(R_1^{(n)'}\right) + \check{p}\left(R_1^{(n)'}\right)}{2} - p^{(n)'}\right), \tag{18}$$

where A_p is a nonnegative quantity given in Appendix 2. Given the relative frequency of I_1 after migration ($R_1^{(n)'}$), larval viability is maximal at an intermediate overall frequency of infection ($p^{(n)'} = (\check{p}(R_1^{(n)'}) + \hat{p}(R_1^{(n)'}))/2$, which can readily be shown to be a maximum, not a minimum or saddle point of $v^{(n)}$). Thus, if higher relative frequency of the stronger strain after migration happens to be associated with extreme (either high or low) overall frequency of infection, it need not be associated with higher larval viability.

At this juncture, simulation is instructive (software available upon request). Figure 1 presents examples of deterministic CI evolution with local density regulation. The strains have equal transmission efficiency and relative fecundity, hence effective

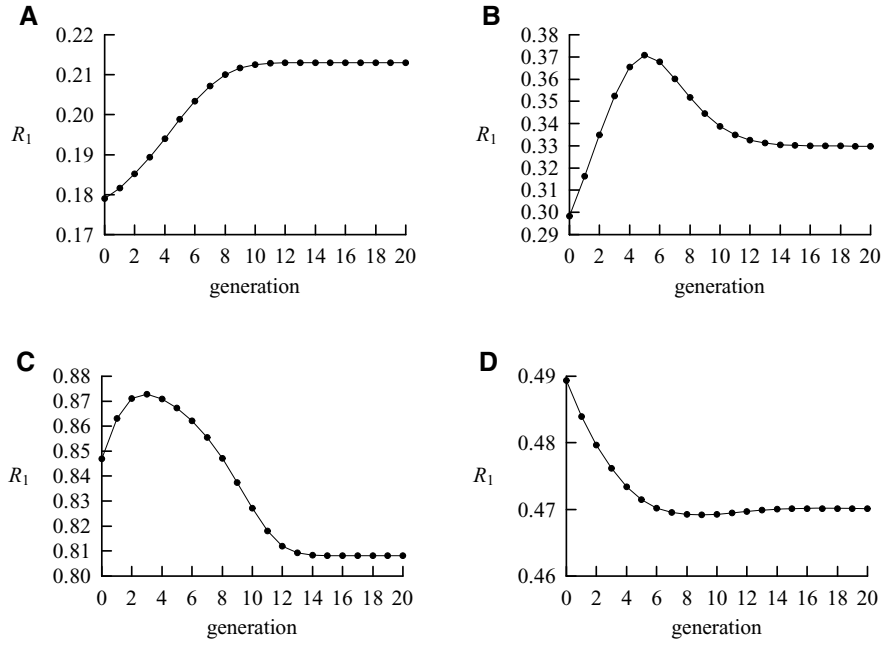

Figure 1. Examples of deterministic CI evolution in subdivided populations with local density regulation. $H_1 = 0$, $H_2 = 0.5$, $\mu_1 = \mu_2 = 0.05$, $F_1 = F_2 = 1$, and $m = 0.05$. In each example, the initial condition of the population is chosen from several generated at random. (A) Two patches, migration before mating, and stepping-stone connectivity. (B) Three patches, migration before mating, and migrant-pool connectivity. (C) Four patches, migration after mating, and stepping-stone connectivity. (D) Five patches, migration after mating, and migrant-pool connectivity.

fecundity, and I_1 has twice the CI intensity of I_2. The number of patches varies from two to five. The examples represent migration both before and after mating and both stepping-stone and migrant-pool connectivity. In each example, the initial condition (i.e., the initial values of $p^{(n)}$ and $R_1^{(n)}$ for each n) is chosen from several generated at random. All the behaviors in these examples are common, and they suggest the range of possibilities. In general, transient evolution gives way to asymptotic stasis. This is unremarkable, because migration homogenizes the patches. More interesting is that for some initial conditions, the initial change in R_1 is positive, but for many others, it is negative, and the same holds for the cumulative change. Thus, the direction of CI evolution depends on the initial condition.

One initial condition of particular interest is that of a population at equilibrium for infection with one strain into which another strain of greater CI intensity is introduced at low frequency. We illustrate this using the strains, patches, and simulator of Figure 1, with two patches, migration before mating, and stepping-stone connectivity. If initially $p_1^{(1)} = 0$, $p_2^{(1)} = \hat{p}(0) = 0.9407373$, $p_1^{(2)} = 0.001$, and $p_2^{(2)} = \hat{p}(0) - p_1^{(2)} = 0.9397373$, then equilibrium quickly ensues, with $p_1^{(1)} = p_1^{(2)} = 0.0005005$ and $p_2^{(1)} = p_2^{(2)} = 0.9403035$, that is, there is a very slight increase in the relative frequency of the stronger strain. This is consistent with Frank's (1997) argument that stronger CI is favored in a subdivided population that is near equilibrium. However, because migration quickly homogenizes the patches, the benefit to I_1 of its greater CI intensity is very slight. Nonetheless, this example suggests that if some mechanism maintained variation among patches, there could be cumulative, directional CI evolution. Demographic fluctuations are a likely mechanism.

Deterministic analysis with systematic outbreeding or inbreeding within patches

In this subsection, we show that systematic outbreeding or inbreeding within patches of a subdivided population can substantially affect CI evolution induced by variation among patches, but whether this favors stronger incompatibility depends on the initial condition of the population.

Over a single generation within a single patch of a subdivided population, sib-mating avoidance and preferential sib-mating have the effects described above for a panmictic population: sib-mating avoidance leads to a larger and preferential sib-mating to a smaller increment to the local overall frequency of infection, but neither affects the local relative frequencies of the strains. The magnitude of the effect on the increment to $p^{(n)}$ may vary among patches, because it depends on $p^{(n)}$ and $R_1^{(n)}$ (note the dependence on p_1 and p_2 in (8a) and (8b)). This affects CI evolution induced by variation among patches, but the consequences depend on the initial condition. The dependence is resistant to algebraic exploration, but simulation readily shows that it can be

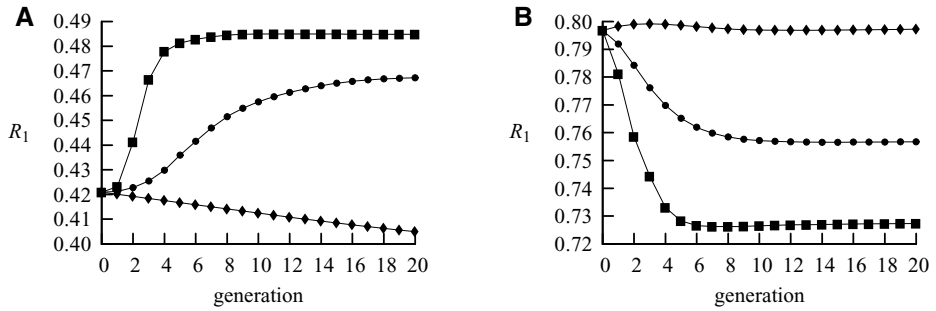

Figure 2. Examples of deterministic CI evolution in subdivided populations with local density regulation and ●—random mating, ■—complete sib-mating avoidance, or ♦—strong preferential sib-mating within patches. As in Figure 1, $H_1 = 0$, $H_2 = 0.5$, $\mu_1 = \mu_2 = 0.05$, $F_1 = F_2 = 1$, and $m = 0.05$. There are two broods per patch. The initial conditions, one for (A) and another for (B), are chosen from several generated at random. Every trajectory represents six patches, migration after mating, and stepping-stone connectivity. (A) For this initial condition, systematic outbreeding favors and inbreeding disfavors the stronger strain, whereas (B) for this initial condition, the opposite holds.

substantial (software available upon request; the representation of nonrandom mating is essentially that of Engelstädter et al. 2006).

Figure 2 presents a pair of examples in which life cycle, connectivity, and parameter values are the same, but the initial condition differs. For one initial condition (1), systematic outbreeding favors and inbreeding disfavors the stronger strain, whereas for the other initial condition (2), the opposite holds. Neither behavior is unusual, nor is either behavior exclusive to higher or lower values of p or R_1. In these examples, the outbreeding is complete sib-mating avoidance: a female always mates with a male from outside her natal brood. The inbreeding is strong preferential sib-mating: a female mates with a male from within her natal brood 85% of the time. (Complete sib-mating would suppress the spread of both strains in these examples. For comparative purposes, we have chosen to consider strong but incomplete sib-mating, so that the overall frequency of infection is increasing in every case.) There are two broods per patch, maximizing the effects of nonrandom mating. With more broods per patch, the contrasts to random mating are qualitatively similar but quantitatively smaller.

Stochastic effects: CI evolution arising from demographic fluctuations

In this subsection, we show that in a finite subdivided population, variation among patches maintained by demographic fluctuations induces CI evolution tending to favor stronger incompatibility; in effect, genetic drift is biased toward stronger CI. However, unless patches are very small, this effect is very weak; even a very small difference of effective fecundity can offset it.

In a subdivided population, migration destroys variation among patches. However, in a finite population, demographic fluctuations continually create variation among patches, leading to persistent heterogeneity. There are fluctuations at three points in the life cycles we consider: (1) migration—infection frequencies among emigrants from a patch typically differ from those in the patch, and for migrant-pool connectivity, infection frequencies among immigrants to a patch typically differ from those in the migrant pool; (2) mating—within each patch, cross frequencies typically differ from their expected values, which are products of infection frequencies; and (3) maturation—within each patch, infection frequencies among adults typically differ from those among larvae. Assuming that every infected female mates, fluctuations in mating cannot induce CI evolution, because the number of infected eggs an infected female lays does not depend on the infection status of her mate. However, fluctuations in migration or maturation create variation in $R_1^{(n)\prime}$ and hence may induce change in R_1, according to (14).

A combination of algebraic analyses and numerical examples yields insights into the effects of these sources of variation, particularly (1). Of primary interest is what ensues once migration has largely homogenized the patches, so suppose that the initial condition of every patch is the nonzero stable deterministic equilibrium. Over the first generation, this situation is algebraically tractable if migration precedes mating and involves only a small fraction of the population. In Appendix 3, we show that for weak migration before mating and stepping-stone connectivity, the expected change in R_1 over one generation is approximately

$$E(\Delta R_1) \approx B_e[F_1(1-\mu_1) - F_2(1-\mu_2)] + \frac{B_h}{K}\left(s_{h_1} - s_{h_2}\right) + \frac{B_f}{K}(F_2\mu_2 - F_1\mu_1), \quad (19)$$

where B_e, B_h, and B_f are nonnegative quantities independent of K and given in Appendix 3. The first term represents selection for higher effective fecundity, and the second and third terms represent the mean effects of demographic fluctuations, namely, selection for stronger incompatibility and for infected females to lay fewer uninfected eggs. If m does not exceed about 0.1, this approximation seems to be fairly accurate. Figure 3 presents simulations with $m = 0.05$ demonstrating the

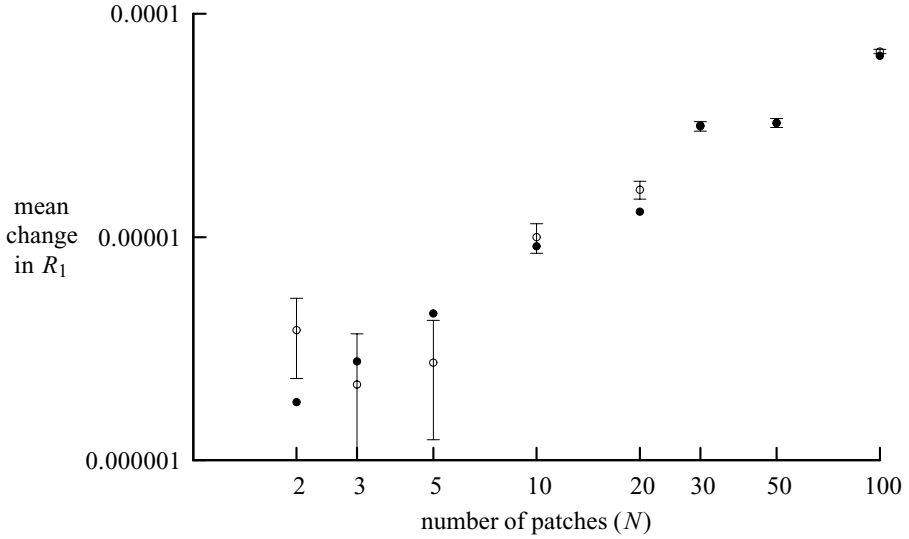

Figure 3. ●—predictions and ○—statistics on single-generation CI evolution arising from demographic fluctuations in subdivided populations with local density regulation. Strain and patch parameters are as in Figures 1 and 2, with the additions that K = nearest_integer(1000/N) and $L = 10$. The initial condition is $p_1^{(n)} = p_2^{(n)}$ = nearest_integer(0.5$\hat{p}$(0.5)K)/K, where $\hat{p}(0.5) = 0.981$. Every datum represents migration before mating and stepping-stone connectivity. Filled circles represent the expected change in R_1 over one generation according to equation (19). Open circles represent the mean over a hundred million simulations of the change in R_1 over one generation. Error bars represent the standard error of the mean. (Irregularities arise from rounding in the initial condition; e.g., for $N = 30$, $p_1^{(n)} = p_2^{(n)} = 0.970$, whereas for $N = 50$, $p_1^{(n)} = p_2^{(n)} = 0.980$.)

accuracy of the second term in particular (software available upon request).

It is reasonable to conjecture that the direction of CI evolution that predominates in the first generation continues to predominate in subsequent generations. However, the population ceases to be homogeneous, so the complex effects of nonuniform initial conditions visible in Figures 1 and 2 might be relevant. Moreover, we have so far restricted our attention to migration before mating and stepping-stone connectivity. Nonetheless, Figure 4 presents simulations over many generations and for all four combinations of life cycle and connectivity that demonstrate cumulative CI evolution favoring stronger incompatibility. The simulator tracks individual hosts, so all three sources of variation discussed above are present (software available upon request). For a given patch size, it makes little difference whether migration is before or after mating or whether connectivity is stepping-stone or migrant-pool. Mean change in R_1 increases as patch size decreases and hence fluctuations become more drastic. Changes over a hundred generations in Figure 4 are more than 100 times the corresponding changes over one generation in Figure 3, implying that migration and demographic fluctuations lead to persistent heterogeneity that favors stronger CI, at least in the cases considered here.

Thus, in a finite subdivided population with local density regulation, CI evolution induced by demographic fluctuations is biased toward stronger incompatibility. However, it should be noted that this bias is weak, and a small difference of effective fecundity can offset it. For example, for migration before mating and stepping-stone connectivity, (19) implies that if

$$F_2(1-\mu_2) - F_1(1-\mu_1) \approx \frac{B_h(s_{h_1} - s_{h_2}) + B_f(F_2\mu_2 - F_1\mu_1)}{B_e K}, \tag{20}$$

$E(\Delta R_1) \approx 0$; in (20) B_e, B_h, and B_f are evaluated at $F_2(1-\mu_2) = F_1(1-\mu_1)$. The offsetting difference is inversely proportional to K, and the coefficient of proportionality may be small. In Figure 4, in simulations of 10 patches, $K = 100$, $B_e = 0.270$, $s_{h_1} - s_{h_2} = 0.5$, $B_h = 0.00183$ and $F_2\mu_2 = F_1\mu_1$, resulting in an offsetting difference of only about 3×10^{-5}.

Discussion

Our analyses show that over a wide range of biologically plausible scenarios, the primary target of selection on alternative, mutually compatible strains of CI-causing bacteria is effective fecundity, $F(1-\mu)$, the product of relative fecundity and bacterial transmission efficiency of infected female hosts, which is proportional to the number of infected progeny an infected female produces. Our results also embody the principle that, all else being equal, a bacterial strain producing stronger CI is favored if and only if the benefit of stronger CI, through reduced larval competition, accrues preferentially to the stronger-CI strain. Host population subdivision per se does not assure this. If infection frequencies

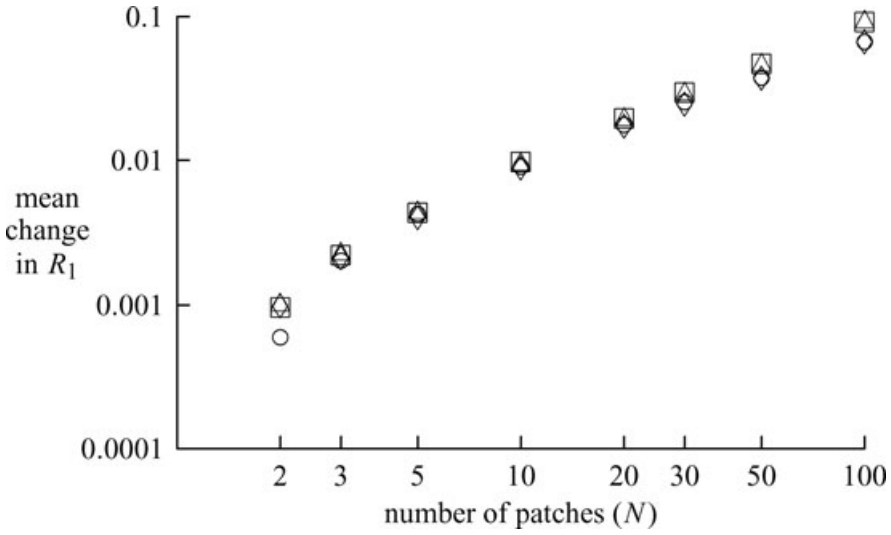

Figure 4. Statistics on multiple-generation CI evolution arising from demographic fluctuations in subdivided populations with local density regulation. Strain and patch parameters and the initial condition are as in Figure 3. Every datum represents the mean over a million simulations of the change in R_1 over a hundred generations for a certain combination of life cycle and connectivity: ○, —migration before mating, stepping-stone connectivity; □, —migration before mating, migrant-pool connectivity; ◇, — migration after mating, stepping-stone connectivity; Δ, —migration after mating, migrant-pool connectivity. The standard error of the mean is less than 1.5×10^{-4} in every case.

vary among patches, so does the relative fitness of infected females. With local density regulation, patches in which the relative fitness of infected females is higher yield more infected progeny, and the relative frequency of whichever strain is more prevalent in them increases. This need not be the stronger-CI strain. Even if it is, this selection for stronger CI is likely to be transient and/or weak.

The relationship between our treatment of host population subdivision and that of Frank (1997, elaborated in Frank 1998, Ch. 7) warrants further comment. As mentioned above, Frank's treatment includes neither an explicit model of population structure nor an explicit analysis of the frequency dynamics of alternative strains. These omissions are potentially problematic, in that although "the kin selection coefficient of relatedness" is treated as a free parameter, it is actually a function of strain frequencies in patches. Readers unaccustomed to this style of analysis may be left with the impression that in subdivided populations with local regulation, selection generally favors stronger CI. However, two limitations of Frank's treatment should be kept in mind. First, it assumes that the overall frequency of infection does not vary among patches. For simplicity, we assumed this as the initial condition in our analysis of stochastic effects, but it does not generally hold (e.g., when a new strain enters a population through migration). Second, Frank's treatment does not capture the changes in the coefficient of relatedness that ensue as strain frequencies change. For example, for two strains in two patches, it can be shown that the coefficient is $(R_1^{(1)} - R_1^{(2)})^2/((R_1^{(1)} + R_1^{(2)})(2 - R_1^{(1)} - R_1^{(2)}))$, which approaches 0 quickly (quadratically) as migration homogenizes the patches (indeed, the coefficient must approach 0 for any numbers of strains and patches). Within its domain of applicability, Frank's analysis agrees with ours (e.g., the deterministic two-patch simulation presented in the "Deterministic analysis with systematic outbreeding or inbreeding within patches" section), but our analyses are more generally applicable.

Overall, competition among mutually compatible strains is probably dominated by selection for higher effective fecundity, for three reasons indicated by our analyses. First, as illustrated in Figure 1, if the initial population is not nearly homogeneous, the strain producing stronger CI need not enjoy an advantage, either initially or cumulatively. In contrast, higher effective fecundity is always favored, regardless of the initial condition. Second, even if the initial population is nearly homogenous, the advantage of the stronger-CI strain is transient, unless some mechanism counteracts migration and maintains variation among patches. In contrast, higher effective fecundity remains favored, even in homogeneous populations. Third, even if some mechanism maintains variation among patches, slightly higher effective fecundity may offset the disadvantage of substantially weaker CI. We have shown this for demographic fluctuations, and it probably holds for other heterogeneity-generating mechanisms such as variation in transmission rates due to local environmental conditions (Stevens and Wicklow 1992; Turelli and Hoffmann 1995). Our results suggest that even in subdivided populations, mod^-resc^+ mutants, which do not induce CI but do protect against it, will spread if they raise effective fecundity.

In a subdivided host population with local density regulation, we have shown that evolution induced by demographic fluctuations is biased toward stronger CI, and this bias strengthens as patch size decreases. Small patches, with intense larval competition, may be common for hosts of CI-causing bacteria, so it is important to consider whether they are likely to engender appreciable selection for stronger CI. Consider, for example, *Drosophila* in an orchard. Mated females lay eggs in fruits, larvae mature inside, and adults emerge, disperse, and mate. The number of females laying eggs in a given fruit may be small; Hoffmann and Nielsen (1985) estimated that two or three females per fruit is typical of *D. melanogaster* in apple orchards. However, two considerations suggest that selection for stronger CI is negligible in such a population. One is that because patches are ephemeral, emigration must be complete, and because distances between patches are small on the scale of adult dispersal distance, the migrant pool is probably well mixed. It can be shown (and is fairly intuitive) that with complete emigration and complete mixing of the migrant pool, genetic drift induced by fluctuations in immigration or maturation is unbiased with respect to CI intensity, assuming mating is within the migrant pool. The other consideration is that population regulation may be largely through losses of entire patches or dispersing adults. Local regulation is crucial to the bias toward stronger CI.

For simplicity, we have supposed that hosts are hermaphroditic. If the sexes are separate, infection frequencies can differ between them, and if mating occurs in small patches, fluctuations in these differences might have a weak systematic effect on CI evolution. This effect may well be negligible in *Drosophila*, where mating probably occurs mainly among dispersing individuals, but it might be appreciable in other hosts. Some hosts may have population structures enabling strong-CI males to reduce larval competition for progeny of their female kin specifically. Such hosts would strongly motivate additional theoretical as well as empirical research on CI evolution.

We have emphasized CI evolution, but our models can be applied to other issues; here are two examples. First, Wade and Stevens (1994) argued that host population subdivision should slow the spread of an infection. However, it is implicit in their treatment that larval viability does not vary among patches. It is easy to see that population subdivision with local regulation can speed the spread of an infection. For example, consider a strain with $H = 0.5$, $\mu = 0.05$, and $F = 1$ infecting a population at $p = 0.25$. If the population is panmictic, regarding the strain as I_1 and applying (5) with $R_1 = 1$ yields $\Delta p = 0.125$. But if the population is evenly divided between two patches with $p^{(1)} = 0.2$ and $p^{(2)} = 0.3$, applying (5) within each patch and averaging the results (a procedure assuming local regulation) yields $\Delta p = 0.130$. (The detailed study by Reuter et al. [2008] of the spatial spread of CI infections likewise concludes that population subdivision will often speed the spread.) Second, Turelli and Hoffmann (1995) observed that in northern California populations of *D. simulans*, p increased from 0.37 in August 1992 to 0.53 in October 1992. Supposing that these populations were isolated, this change demands an implausibly short generation time. However, in a two-patch model ((10)–(12) with $R_1 = 1$) with $H = 0.55$, $\mu = 0.042$ and $F = 1$ (Turelli and Hoffmann's estimates), migration of 5% per generation between a patch initially at $p = 0.94$, the nonzero stable equilibrium value, and a patch initially at $p = 0.37$ drives the latter to $p = 0.56$ in three generations, assuming the patches are of equal size; if the "source" patch is larger, so is the effect. This suggests that the northern California populations were affected by immigration from populations already saturated with the infection. The known presence of such populations to the south lends credence to this idea.

Our results affirm the importance of fecundity and transmission efficiency of infected female hosts for competition between CI-causing bacterial strains. For mutually compatible strains, these traits are probably decisive, whether the host population is panmictic or subdivided. For partially incompatible strains, Turelli (1994) showed that these traits remain significant in panmictic populations, and we expect that the same holds in subdivided populations. Thus, CI evolution depends strongly on relationships among CI intensity, host fecundity, and transmission efficiency. The data of Weeks et al. (2007), revealing *Wolbachia* evolution that has appreciably changed host fecundity but not CI intensity or transmission efficiency, suggest that these relationships need not be simple. These observations are consistent with Frank's (1997) skepticism that CI is a pleiotropic byproduct of bacterial processes that benefit hosts.

These theoretical and empirical findings present an evolutionary conundrum: if selection on CI-causing bacteria primarily favors higher effective fecundity rather than stronger CI, why is CI so common in nature? We find the clade-selection argument of Hurst et al. (1992) and Hurst and McVean (1996) the most plausible proposal to date. Imperfect transmission yields a persistent residuum of uninfected eggs vulnerable to CI. Because the nuclear background is quickly randomized between infected and uninfected hosts (Turelli et al. 1992), this engenders strong selection on hosts to suppress CI (Turelli 1994); comparative data on *Wolbachia* in *D. simulans* and *D. melanogaster* (Hoffmann and Turelli 1997) support this expectation, as do (indirectly) data on the host suppression of the male-killing *Wolbachia* phenotype in the butterfly *Hypolimnas bolina* (Hornett et al. 2006). Host suppression of CI can lead to the elimination of the bacteria, once CI is too weak to offset imperfect transmission and fecundity costs (Hurst and McVean 1996). One route to stable coexistence is the evolution of obligate mutualism, as observed between *Wolbachia* and some nematodes (Hoerauf et al. 1999) and the parasitic wasp *Asobara tabida* (Dedeine et al. 2004; Pannebakker et al. 2007).

However, this does not seem to be a common route for *Wolbachia* evolution in *Drosophila*, where comparative data indicate that *Wolbachia* incidence is dominated by transfer between host species rather than persistence through host speciation (O'Neill et al. 1992; Mateos et al. 2006; Zabalou et al. 2008). This suggests that the prevalence of CI may reflect clade selection favoring bacterial lineages that produce strong CI in novel hosts and hence spread rapidly in them.

Recent meta-analyses relating speciation rates to traits such as sexual dichromatism in birds or polyandry in insects suggest that "species selection," defined as differential diversification rates, may significantly influence macroevolutionary trends in character evolution (Coyne and Orr 2004, ch. 12). CI may be a trait that is selected against within lineages associated with particular hosts but persists owing to the differential proliferation of lineages that undergo successful transfer between hosts more often. A similar argument has been made for the importance of horizontal transmission in the evolutionary persistence of transposable elements (e.g., Burt and Trivers 2006, ch. 7). To understand the relative importance of evolution within versus among host species to the incidence of CI-causing bacteria and the intensity of CI, we need more data regarding phylogenetic relationships of bacteria and hosts (preferably extensive surveys of clades, e.g., Mateos et al. 2006), more data regarding effects of bacteria on hosts (especially longitudinal measurements, e.g., Weeks et al. 2007), more estimates of the ages of bacteria–host associations (Hoffmann and Turelli 1997; Ballard 2004), and estimates of the frequency of horizontal transmission relative to host speciation.

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Appendix 1

ALTERNATIVE MODELS OF POPULATION SUBDIVISION

For migrant-pool (rather than stepping-stone) connectivity, suppose that a fraction m of hosts in each patch is exchanged with the migrant pool. Then (10) is replaced by

$$P_i^{(n)'} = p_i^{(n)} K(1-m) + p_i K m. \qquad \text{(A1)}$$

Following this replacement, (11)–(18) and the accompanying arguments are unaltered.

For migration after mating, (10) or (A1), (11)–(15), and the accompanying arguments are unaltered, but (16)–(18) are replaced by more complicated formulas, because $Q^{(n)''}$ depends on the frequencies of mated females representing all nine types of cross. However, simulation remains straightforward, and our software, including the source code, is available upon request. Comparing examples such as Figure 1A and B (migration before mating) to Figure 1C and D (migration after mating) suggests that the order of migration and mating per se makes little difference. However, systematic outbreeding or inbreeding may be more prevalent in species in which migration follows mating. For this reason, the examples in Figure 2 feature this life cycle.

Appendix 2

FACTORS APPEARING IN EQUATIONS (17) AND (18)

For migration before mating and stepping-stone connectivity, (11), (15), (16) and routine calculus and algebra yield

$$\frac{\partial v^{(n)}}{\partial R_1^{(n)'}} = A_\varphi[F_2(1-\mu_2) - F_1(1-\mu_1)] + A_h(s_{h_1} - s_{h_2}) + A_f(F_2\mu_2 - F_1\mu_1), \quad \text{(A2)}$$

where

$$A_\varphi = \frac{K' f L \left(v^{(n)}\right)^2 p^{(n)'}}{K}, \quad \text{(A3a)}$$

$$A_h = A_\varphi\left[p^{(n)'}\left(R_1^{(n)'}F_1\mu_1 + R_2^{(n)'}F_2\mu_2\right) + q^{(n)'}\right], \text{ and} \quad \text{(A3b)}$$

$$A_f = A_\varphi\left\{p^{(n)'}\left[1 - \left(R_1^{(n)'}s_{h_1} + R_2^{(n)'}s_{h_2}\right)\right] + q^{(n)'}\right\}. \quad \text{(A3c)}$$

Similarly, the factor A_p that appears in (18) is

$$A_f = \frac{A_\varphi\left(R_1^{(n)'}s_{h_1} + R_2^{(n)'}s_{h_2}\right)\left[1 - \left(R_1^{(n)'}F_1\mu_1 + R_2^{(n)'}F_2\mu_2\right)\right]}{p^{(n)'}}. \quad \text{(A4)}$$

Appendix 3

DERIVATION OF EQUATION (19)

Suppose that initially $p_1^{(n)} = p_1$ and $p_2^{(n)} = p_2$ for each n. For migration before mating, the first event in the life cycle is migration. For stepping-stone connectivity, the frequency of I_i in patch n after migration is

$$p_i^{(n)'} = \frac{p_i K - M_{\ell,i}^{(n)} - M_{r,i}^{(n)} + M_{r,i}^{(\ell(n))} + M_{\ell,i}^{(r(n))}}{K} = p_i + \frac{M_i^{(n)}}{K}, \quad \text{(A5)}$$

where $M_{\ell,i}^{(n)}$ and $M_{r,i}^{(n)}$ are the numbers of I_i hosts that migrate from patch n to patches $\ell(n)$ and $r(n)$, respectively, and

$$M_i^{(n)} = -M_{\ell,i}^{(n)} - M_{r,i}^{(n)} + M_{r,i}^{(\ell(n))} + M_{\ell,i}^{(r(n))} \quad \text{(A6)}$$

is a convenient abbreviation; $M_{\ell,i}^{(n)}$, $M_{r,i}^{(n)}$, and $M_i^{(n)}$, are random variables. The next events are mating and hatching. The frequency of I_i in patch n after hatching is

$$p_i^{(n)''} = \frac{p_i^{(n)'}\varphi_i}{\overline{W}^{(n)'}}, \quad \text{(A7)}$$

where $\varphi_\iota = F_i(1-\mu_i)$ is a convenient abbreviation and

$$\overline{W}^{(n)'} = p_1^{(n)'}\varphi_1 + p_2^{(n)'}\varphi_2 + \left(p_1^{(n)'}F_1\mu_1 + p_2^{(n)'}F_2\mu_2 + q^{(n)'}\right) \times \left(p_1^{(n)'}H_1 + p_2^{(n)'}H_2 + q^{(n)'}\right); \quad \text{(A8)}$$

sampling deviations in mating are neglected, because they have no mean effect over one generation. By (2), (12), (14), and routine algebra,

$$\overline{W}^{(n)'} = W - \frac{L^{(n)}}{K} - \frac{Q^{(n)}}{K^2}, \text{ where} \quad \text{(A9)}$$

$$L^{(n)} = \left(M_1^{(n)}s_{f_1} + M_2^{(n)}s_{f_2}\right) + \left[M_1^{(n)}(F_1\mu_1 - 1) + M_2^{(n)}(F_2\mu_2 - 1)\right]p\overline{s_h} + \left(M_1^{(n)}s_{h_1} + M_2^{(n)}s_{h_2}\right)\left[1 - p(1-\overline{F\mu})\right] \text{ and} \quad \text{(A10a)}$$

$$Q^{(n)} = \left[M_1^{(n)}(F_1\mu_1 - 1) + M_2^{(n)}(F_2\mu_2 - 1)\right] \times \left(M_1^{(n)}s_{h_1} + M_2^{(n)}s_{h_2}\right). \quad \text{(A10b)}$$

are linear and quadratic, respectively, in $M_1^{(n)}$and $M_2^{(n)}$. Applying the geometric series formula to (A7) and neglecting terms of order greater than two in $M_1^{(n)}$and $M_2^{(n)}$yields

$$p_i^{(n)''} \approx \frac{\varphi_i}{\overline{W}}\left(p_i + \frac{M_1^{(n)}}{K}\right)\left(1 + \frac{L^{(n)}}{\overline{W}K} + \frac{Q^{(n)}}{\overline{W}K^2} + \frac{\left(L^{(n)}\right)^2}{\overline{W}^2K^2}\right) \quad \text{(A11a)}$$

$$\approx \frac{\varphi_i}{\overline{W}}\left(p_i + \frac{M_i^{(n)}}{K} + \frac{p_iL^{(n)}}{\overline{W}K} + \frac{M_i^{(n)}L^{(n)}}{\overline{W}K^2} + \frac{p_iQ^{(n)}}{\overline{W}K^2} + \frac{p_i\left(L^{(n)}\right)^2}{\overline{W}^2K^2}\right). \quad \text{(A11b)}$$

The next event is maturation. The frequency of I_i in patch n after maturation is

$$p_i^{(n)'''} = p_i^{(n)''}; \quad \text{(A12)}$$

sampling deviations in maturation are neglected, because they have no mean effect over one generation. Thus, the frequency of I_i in the entire population after a generation is

$$p_i''' = \frac{\sum_n p_i^{(n)''}}{N} \approx \frac{\varphi_i}{\overline{W}} \times \left(p_i + \frac{\sum_n M_i^{(n)}L^{(n)}}{\overline{W}K^2N} + \frac{p_i\sum_n Q^{(n)}}{\overline{W}K^2N} + \frac{p_i\sum_n (L^{(n)})^2}{\overline{W}^2K^2N}\right), \quad \text{(A13)}$$

using the fact that $\sum_n M_i^{(n)} = 0$, hence $\sum_n L^{(n)} = 0$. The relative frequency of I_1 in the entire population after a generation is

$$R_i''' = \frac{p_1'''}{p_1''' + p_2'''}. \tag{A14}$$

Applying the geometric series formula to (A14) and neglecting terms of order greater than two in $M_1^{(n)}$and $M_2^{(n)}$yields

$$R_1''' \approx \frac{p_1\varphi_1}{p_1\varphi_1 + p_2\varphi_2}$$

$$\times \left(1 + \frac{\sum_n M_1^{(n)} L^{(n)}}{p_1 \overline{W} K^2 N} + \frac{\sum_n Q^{(n)}}{\overline{W} K^2 N} + \frac{\sum_n \left(L^{(n)}\right)^2}{\overline{W}^2 K^2 N}\right) \tag{A15a}$$

$$\times \left(1 - \frac{\sum_n \left(M_1^{(n)}\varphi_1 + M_2^{(n)}\varphi_2\right) L^{(n)}}{(p_1\varphi_1 + p_2\varphi_2)\overline{W} K^2 N} - \frac{\sum_n Q^{(n)}}{\overline{W} K^2 N} - \frac{\sum_n \left(L^{(n)}\right)^2}{\overline{W}^2 K^2 N}\right)$$

$$\approx \frac{p_1\varphi_1}{p_1\varphi_1 + p_2\varphi_2}\left(1 + \frac{\varphi_2 \sum_n \left(p_2 M_1^{(n)} - p_1 M_2^{(n)}\right) L^{(n)}}{p_1(p_1\varphi_1 + p_2\varphi_2)\overline{W} K^2 N}\right). \tag{A15b}$$

Thus,

$$\Delta R_1 \approx \frac{R_1 R_2(\varphi_1 - \varphi_2)}{R_1\varphi_1 + R_2\varphi_2} + \frac{\varphi_1\varphi_2 \sum_n p_2 M_1^{(n)} L^{(n)} - p_1 M_2^{(n)} L^{(n)}}{p^2(R_1\varphi_1 + R_2\varphi_2)^2 \overline{W} K^2 N}. \tag{A16}$$

Assuming that m is small, the joint distribution of $M_{\ell,1}^{(n)}$ and $M_{\ell,2}^{(n)}$ or $M_{r,1}^{(n)}$ and $M_{r,2}^{(n)}$ is approximately the trinomial distribution with parameters $Km/2$, p_1, and p_2, hence by (A6) and standard results,

$$E\left(M_i^{(n)}\right) = 0,\ E\left(\left(M_i^{(n)}\right)^2\right) \approx 2Kmp_i(1 - p_i),\ \text{and}$$
$$E\left(M_1^{(n)} M_2^{(n)}\right) \approx -2Kmp_1p_2. \tag{A17}$$

By these and routine algebra, the expected value of the sum in (A16) is

$$\frac{2Nmp^2 R_2 R_2}{K}\{[1 - p(1 - \overline{F\mu})](s_{h_1} - s_{h_2}) + (1 - p\overline{s_h})(F_2\mu_2 - F_1\mu_1)\}. \tag{A18}$$

Thus,

$$E(\Delta R_1) \approx B_e(\varphi_1 - \varphi_2) + \frac{B_h}{K}(s_{h_1} - s_{h_2}) + \frac{B_f}{K}(F_2\mu_2 - F_1\mu_1), \tag{A19}$$

where

$$B_e = \frac{R_1 R_2}{R_1\varphi_1 + R_2\varphi_2}, \tag{A20a}$$

$$B_h = \frac{2mR_1\varphi_1 R_2\varphi_2(1 - p(1 - \overline{F\mu}))}{(R_1\varphi_1 + R_2\varphi_2)^2\overline{W}},\ \text{and} \tag{A20b}$$

$$B_f = \frac{2mR_1\varphi_1 R_2\varphi_2(1 - p\overline{s_h})}{(R_1\varphi_1 + R_2\varphi_2)^2\overline{W}} \tag{A20c}$$

If $N = 1$, (A17), (A18), (A20b), and (A20c) are incorrect, because it is implicit in (A17) that emigrants from and immigrants to patch n are distinct; instead, $B_h = B_f = 0$.