Symposium

The Ecology and Evolution of Animal Medication: Genetically Fixed Response versus Phenotypic Plasticity*

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q1 abstract: Animal medication against parasites can occur either as a genetically fixed (constitutive) or phenotypically plastic (induced) behavior. Taking the tritrophic interaction between the monarch butterfly *Danaus plexippus*, its protozoan parasite *Ophryocystis elektroscirrha*, and its food plant *Asclepias* spp. as a test case, we develop a game-theory model to identify the epidemiological (parasite prevalence and virulence) and environmental (plant toxicity and abundance) conditions that predict the evolution of genetically fixed versus phenotypically plastic forms of medication. Our model shows that the relative benefits (the antiparasitic properties of medicinal food) and costs (side effects of medicine, the costs of searching for medicine, and the costs of plasticity itself) crucially determine whether medication is genetically fixed or phenotypically plastic. Our model suggests that animals evolve phenotypic plasticity when parasite risk (a combination of virulence and prevalence and thus a measure of the strength of parasite-mediated selection) is relatively low to moderately high and genetically fixed medication when parasite risk becomes very high. The latter occurs because at high parasite risk, the costs of plasticity are outweighed by the benefits of medication. Our model provides a simple and general framework to study the conditions that drive the evolution of alternative forms of animal medication.

Keywords: self-medication, phenotypic plasticity, zoopharmacognosy, ecological immunology, behavioral immunity, disease ecology.

Introduction: Ecology and Evolution of Animal Medication

Animal hosts have evolved a wide array of defenses against their parasites: animals may either avoid parasite infection, reduce parasite growth on infection, or tolerate the effects of infection without reducing parasite burdens (Boots and Bowers 1999; Rolff and Siva-Jothy 2003; Råberg et al. 2007; Michalakis 2009; Hayward et al. 2014). Although many of these defenses are based on highly evolved cellular and humoral immune mechanisms, it is becoming increasingly clear that animals may also employ behaviors as defenses against their parasites (Hart 1990, 2005; Moore 2002; Hutchings et al. 2003, 2006; de Roode and Lefèvre 2012). For example, gypsy moth larvae avoid foliage with virus-killed cadavers (Capinera et al. 1976; Parker et al. 2010), locusts increase their body temperature by seeking out warmer environments to reduce parasitic fungal growth (Elliot et al. 2002), and crickets increase their egg-laying rate in response to bacterial infection (Adamo 1999).

One way in which animals defend themselves against parasites is by using medication, defined as the use of third species or compounds to prevent, reduce, or tolerate parasite infection (Clayton and Wolfe 1993; Lozano 1998; Huffman 2003; de Roode et al. 2013). Animal medication has traditionally been studied in large mammals, such as primates and sheep (Janzen 1978; Wrangham and Nishida 1983; Huffman and Seifu 1989; Huffman et al. 1993, 1996, 1997; Huffman 1997, 2003; Fowler et al. 2007; Villalba et al. 2010), but recent studies in insects indicate that medication is much more widespread than initially thought (Lee et al. 2006; Povey et al. 2009; Singer et al. 2009; Lefèvre et al. 2010; de Roode and Lefèvre 2012). Animal medication is often categorized into two distinct forms (e.g., Lozano 1991, 1998). Therapeutic medication is used by infected individuals in response to parasite infection. On the other hand, prophylaxis is used by infected and uninfected individuals alike and does not depend on actual parasite infection. It has been hypothesized that the relative risks imposed by parasites—compared to the potential toxicity of medication—may importantly determine whether animals use prophylaxis or therapeutic medication. In particular, when parasite risk is high (meaning that parasite-mediated selection is high) and predictable spatially or

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seasonally, prophylaxis may be favored. In contrast, when parasite risk is low or variable, therapeutic responses may be more favorable, since animals would use medication only when needed (Phillips-Conroy 1986; Lozano 1991, 1998; Castella et al. 2008).

Animals may use prophylaxis and therapeutic medication to either treat themselves (self-medication) or their genetic kin (kin medication). For example, woolly bear caterpillars infected with parasitoid flies increase their ingestion of alkaloids, which are detrimental to the flies (Singer et al. 2009): a case of therapeutic self-medication. In contrast, wood ants and honeybees incorporate antimicrobial resin into their nests, which reduces parasite growth, and thereby protects the whole colony: a case of prophylactic kin medication (Christe et al. 2003; Chapuisat et al. 2007; Castella et al. 2008; Simone et al. 2009; Simone-Finstrom and Spivak 2010).

Because animal medication is common and because it has major effects on host and parasite fitness, it is likely to play a major role in the ecology and evolution of disease (de Roode et al. 2013). For example, by providing partial resistance to parasite infection, animal medication could select for parasites with higher rates of intrinsic virulence (de Roode et al. 2011*a*), similar to the effects of genetically determined immunity or imperfect vaccines (Gandon and Michalakis 2000; Gandon et al. 2001). In addition, animals may adapt to their parasites using medicine, such that hosts and parasites may locally adapt to medicinal factors (de Roode et al. 2013), in addition to each other's genotypes (e.g., Koskella 2014; Vergara et al. 2014). Moreover, by reducing parasite infection, virulence and transmission, animal medication is likely to have strong effects on disease epidemiology (Foster et al. 1992), similar to the effects of other community members on host-parasite interactions (e.g., Auld et al. 2014).

Despite the importance of animal medication for host-parasite biology, its evolution is still poorly understood. There are essentially two main ways in which animal medication may evolve: medication can either be a genetically fixed trait or a phenotypically plastic response. Fixed medication occurs when an animal always uses the medication behavior, regardless of actual infection and regardless of infection risk, such as occurs with wood ants that collect antimicrobial resin (Castella et al. 2008). In contrast, a plastic form of medication is one by which an animal uses medication only when it is infected or when it perceives an increased risk of parasitism, such as occurs with the increased alkaloid ingestion of woolly bear caterpillars infected with parasitoid flies (Singer et al. 2009).

Here we will develop a simple game-theory approach to investigate the conditions under which genetically fixed and phenotypically plastic forms of medication may evolve (see Lively 1986 and Moran 1992 for similar approaches on the evolution of plasticity in spatially variable environments). We have little insight into this question, although some predictions can be made. In particular, it could be argued that high parasite risk (which could be determined by both high parasite prevalence and/or high virulence) should select for genetically fixed medication, while low and variable risk should select for phenotypically plastic medication. This prediction is based on the costs that are associated with medication behaviors. For example, the alkaloids that woolly bear caterpillars use to fight off parasitoid fly infection also reduce the survival of uninfected caterpillars (Singer et al. 2009). Given such costs, the evolution of plasticity could be expected because plasticity would ensure animals to benefit from medicine when infected, but to not pay the costs when uninfected. However, plasticity itself may be costly to maintain (DeWitt et al. 1998; Agrawal 2001; Mooney and Agrawal 2007), partly due to the maintenance of the physiological and genetic mechanisms that are required for animals to alter their behavior in response to parasite infection or risk. Thus, it could be predicted that when parasite risk is high, the costs of plasticity (which are always paid) may outweigh the costs of medication, and a fixed strategy may be favored over a plastic response.

In order to make more formal predictions, we develop a game-theory mathematical model and build on the insights obtained from a specific system of monarch butterflies (*Danaus plexippus*) and their protozoan parasites, in which kin medication has been demonstrated previously (Lefèvre et al. 2010, 2012). Our aims are to (1) provide a general framework to study the conditions that drive the evolution of alternative forms of animal medication and (2) generate specific hypotheses that can be tested in the monarch butterfly system, as well as other systems.

Animal Medication in Monarch Butterflies and Their Protozoan Parasites

Monarch butterflies are best known for their spectacular annual migration from the United States and Canada to their overwintering sites in Mexico (Urquhart and Urquhart 1978; Brower 1995), but they also occur in nonmigratory populations around the world (Ackery and Vane-Wright 1984). In all these populations, monarchs are commonly infected with the protozoan parasite *Ophryocystis elektroscirrha* (Leong et al. 1997; Altizer and de Roode, forthcoming). *Ophryocystis elektroscirrha* is an apicomplexan parasite that forms dormant spores on the outside of the monarch butterfly (McLaughlin and Myers 1970). Most parasite transmission occurs when females scatter spores onto the eggs and larval food plants on which they oviposit (de Roode et al. 2009): spores that are ingested by hatching caterpillars release sporozoites in the

mid gut that subsequently invade the larval hypoderm. The parasite then successively replicates asexually during the larval and pupal stages and sexually during the pupal stage, and newly emerged butterflies carry up to millions of parasite spores on the outsides of their bodies (de Roode et al. 2007). These high parasite loads facilitate *O. elektroscirrha* transmission but are detrimental to monarchs, with high parasite loads resulting in reduced preadult survival, adult mating ability, fecundity, flight ability, and life span (Bradley and Altizer 2005; de Roode et al. 2008*b*, 2009).

Although monarch adults are generalist feeders on flower nectar, monarch caterpillars are specialist feeders on milkweeds, mostly in the genus *Asclepias* (Ackery and Vane-Wright 1984; Malcolm 1991). As with many other tritrophic interactions between plants, insects and their parasites (Cory and Hoover 2006), milkweeds are a major determinant of monarch resistance and parasite virulence (Sternberg et al. 2012; see fig. 1*A*). For example, monarchs reared on the tropical milkweed *Asclepias curassavica* suffer much lower parasite growth and virulence than monarchs reared on the swamp milkweed *Asclepias incarnata* (de Roode et al. 2008*a*). Milkweeds are well known to contain a class of secondary toxic chemicals, called cardiac glycosides or cardenolides, and milkweeds vary greatly in their cardenolide concentration and composition (Malcolm and Brower 1989; Malcolm 1991). Experiments have suggested that milkweeds with higher concentrations of cardenolides are more medicinal (de Roode et al. 2011*b*; Sternberg et al. 2012; see fig. 1*B*), although high concentrations can also result in detrimental side effects (Zalucki et al. 2001*a*, 2001*b*; Agrawal et al., forthcoming).

Previous studies have shown that monarch butterflies can use medication as a defense against *O. elektroscirrha* (Lefèvre et al. 2010, 2012). Monarch larvae do not preferentially use medicinal milkweed when infected, but adults do. In experiments where female butterflies from the western United States were given the choice between *A. curassavica* and *A. incarnata* plants, infected butterflies strongly preferred to oviposit on the medicinal *A. curassavica*, while uninfected monarchs had no such preference (fig. 1*C*, 1*D*). By laying their eggs on medicinal milkweed, infected monarchs reduce parasite infection, growth, and virulence in their offspring: an example of offspring medication (Lefèvre et al. 2010). Because monarchs preferred to lay eggs on the medicinal milkweed only when infected, this form of medication is phenotypically plastic, not genetically fixed. Moreover, there are clear costs associated with this medication, with uninfected monarchs suffering shorter life spans when reared on the medicinal milkweed (Lefèvre et al. 2010).

Although *O. elektroscirrha* has been detected in all monarch populations studied to date, parasite prevalence varies dramatically between populations. In the eastern and western United States, parasite prevalence ranges from around 2%–20%. In contrast, in south Florida, parasite prevalence ranges from around 80%–100% (Altizer et al. 2000; Altizer and de Roode, forthcoming). In the following sections, we develop a mathematical model to determine whether high parasite risk is indeed expected to select for genetically fixed medication, and provide recommendations for future experiments to validate model predictions. In order to analytically track the mechanisms underlying the evolution of medication, we opted for a simple game-theory model, discarding the possibilities of polymorphism and epidemiological feedback.

Model Development: Plant Preference

Consider a population of butterflies with birth and death rates ν and μ, respectively. Call τ the fitness cost due to plant toxicity, α the cost due to parasite infection (called virulence hereafter), and b ($0 \leq b \leq 1$) the relative decrease in virulence due to the plant toxicity (as observed in experimental results). The latter can be due to lower infection or higher clearance rates. The fitness W_R of the butterfly genotype R that chooses the plant to lay eggs at random reads

$$W_R = \frac{\nu}{\mu + q\tau + (1 - qb)p\alpha}, \quad (1)$$

where q is the proportion of toxic plants in the environment and p is the parasite prevalence. We assume for the purpose of this article that these proportions are parameters held constant. In particular, we ignore any potential epidemiological feedback that could modify the parasite prevalence. In the model analysis, however, we explore the whole ranges of toxic plants proportion and parasite prevalence. Note also that the birth component of the fitness function is the constant ν that does not depend on the type of plant the eggs are laid on. Other fitness functions introduced in the rest of the manuscript are variants of this one. Their assumptions will thus be the same, unless otherwise specified. Table 1 contains the list of all the parameters of the model with their definitions.

The fitness function of equation (1) assumes no butterfly preference for a particular plant type. In this case, the phenotype we are interested in (i.e., the plant type on which the butterfly lay its eggs) is determined solely by the environment (here the proportion q of toxic plants in the environment). In the cases where the butterflies have a preference for a particular plant type—be it genetically fixed or plastic—note that the phenotype we are interested in still depends on the proportion q of toxic plants in the environment. For example, in the extreme case where there

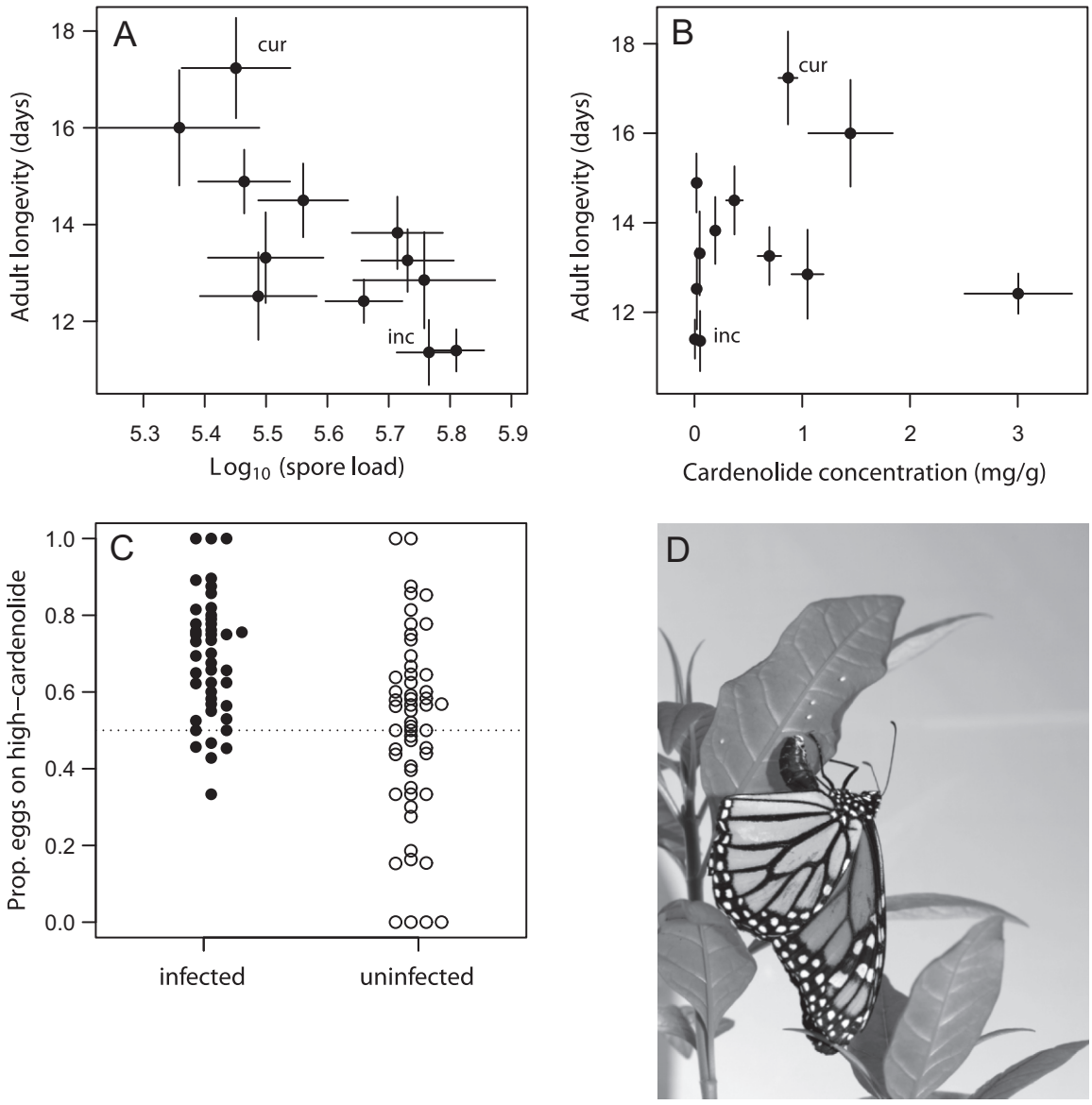

Figure 1: *A*, Variation in parasite spore load and adult longevity experienced by monarchs reared on 12 different species of milkweed (Sternberg et al. 2012); inc and cur denote *Asclepias incarnata* and *Asclepias curassavica*, respectively. *B*, Across 12 species of milkweed, higher cardenolide concentrations initially increase the adult longevity of infected monarchs but then decrease it again at very high levels of cardenolides (Sternberg et al. 2012). *C*, The proportion of eggs laid on the high-cardenolide *A. curassavica* when provided a two-way choice between *A. curassavica* and *A. incarnata* plants by infected and uninfected monarch butterflies derived from California (Lefèvre et al. 2010). *D*, A monarch laying eggs on *A. curassavica*. Data points and error bars in *A* and *B* denote species means and standard errors; data points in *C* denote individual butterflies.

are only toxic plants in the environment, even a butterfly intending to lay eggs on nontoxic plants will have no choice but to lay eggs on the toxic ones. This is what is meant, in this article, by preference, as opposed to specialization where a butterfly would be able to lay eggs on only one type of plant, regardless its availability. In order to account for both the plant availability in the environment and the strength of plant preference, we introduce the probabilities $g(q)$ and $g(1 - q)$ that butterflies respectively intending to lay eggs on toxic and nontoxic plants actually do so. The function g should be a convex function with constraints $g(0) = 0$ and $g(1) = 1$ and with the magnitude of convexity determining the plant preference. A simple example of such a function could be

Table 1: Parameters of the model and their definitions

Parameter	Definition
ν	Birth rate
μ	Death rate
τ	Cost of plant toxicity to the monarch
b	Cost of plant toxicity to the parasite ($0 \le b \le 1$)
q	Proportion of toxic plants in the environment
p	Parasite prevalence
α	Parasite virulence
ε	Strength of plant preference
c	Cost of plant preference
φ	Cost of phenotypic plasticity

$$g(q) = 1 - (1 - q)^{\varepsilon}, \tag{2}$$

for which we can verify that the convexity increases with the strength of plant preference ε ($\varepsilon \ge 1$). See figure 2*A* for the shape of equation (2) for different values of plant preference ε. Absence of preference corresponds to the particular case where $\varepsilon = 1$. Note that the particular function of equation (2) for the plant preference will be used for illustrative purposes only and that the general results of the model will not depend on the specific shape of the preference function g, beside being convex and with constraints $g(0) = 0$ and $g(1) = 1$. We will hereafter call $g = g(q)$ the probability that butterflies intending to lay eggs on toxic plants actually do so and $f = g(1 - q) = 1 - q^{\varepsilon}$ the probability that butterflies intending to lay eggs on nontoxic plants actually do so. We will furthermore assume that these probabilities g and f do not depend on the determinism of preference (i.e., fixed or plastic).

Two properties of function g, both a consequence of convexity, will be used in the following sections. The first one is that

$$0 \le g(q) - q \le 1, \forall q \in [0, 1]. \tag{3}$$

The difference $g(q) - q$ quantifies the efficiency in picking up toxic plants, as compared to a random choice that would depend only on the availability q of the toxic plants in the environment. This quantity can thus be interpreted as the strength of the selective pressure in favor of plant preference. Similarly, $g(1 - q) - (1 - q)$ quantifies the efficiency in picking up nontoxic plants. Using the example of equation (2), figure 2*B* illustrates how this efficiency depends on both the plant preference ε and the proportion q of toxic plants in the environment. As expected, in absence of any possible choice ($q = 0$ or $q = 1$), the efficiency is null, whatever the level of plant preference ε. Between these two extremes, the efficiency reaches a maximum value. Figure 2*B* shows that, as expected, the efficiency $g(q) - q$ in picking up a toxic plant increases with the preference ε for toxic plants, whatever the availability of q of toxic plants in the environment. Also as expected, it is for low proportions of toxic plants in the environment that this increase of efficiency with plant preference is the most pronounced, as materialized by the shift of the mode toward the low q values (dashed line in fig. 2*B*). Exactly the same property can be derived for the preference for nontoxic plants in the environment. The second property of the preference function is that

$$g + f - 1 = g(q) + g(1 - q) - 1 \ge 0, \forall q \in [0, 1] \tag{4}$$

and is a direct consequence of the first property. It has no particular biological interpretation but this mathematical

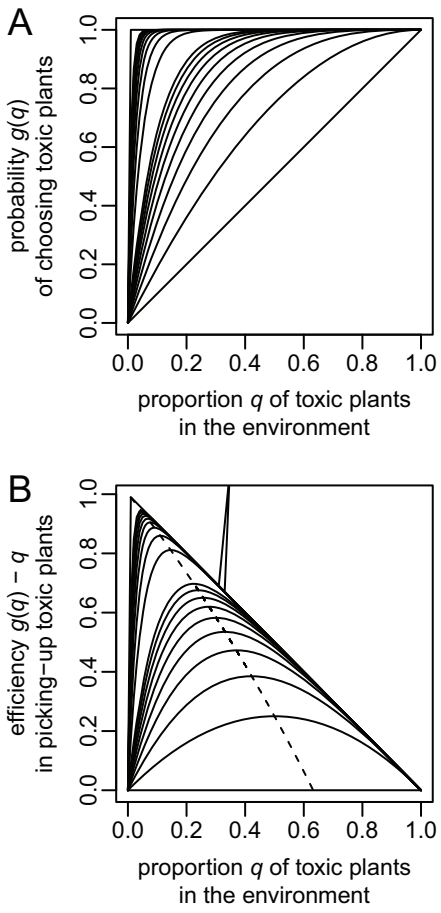

Figure 2: Probability $g(q)$ of choosing a toxic plant (*A*) and efficiency $g(q) - q$ in picking up a toxic plant (*B*) as functions of the proportion q of toxic plants in the environment. Here, $g(q)$ is defined according to equation (2). From bottom to top, the first 10 lines have values of plant preference ε rising from 1 to 10 by steps of 1, the next 9 lines have values of plant preference ε rising from 20 to 100 by steps of 10, and the last line has a plant preference $\varepsilon = 1{,}000$. *B*, The dashed line shows the maxima of the efficiency curves. Similar curves are obtained for the probability $g(1 - q) = 1 - q^{\varepsilon}$ of choosing a nontoxic plant and efficiency $g(1 - q) - (1 - q) = q - q^{\varepsilon}$ in picking up a nontoxic plant, as functions of the proportion $1 - q$ of nontoxic plants in the environment.

property will be used in interpreting equations (11) and (12).

Modeling Fixed Genetic Determinism

Let us now consider the case where the butterfly population is made of two genotypes: genotype T, which preferentially lays eggs on toxic plants and thus has a fitness

$$W_{\mathrm{T}} = \frac{\nu}{\mu + g\tau + (1 - gb)p\alpha + c}, \tag{5}$$

and genotype N, which preferentially lays eggs on nontoxic plants and thus has a fitness

$$W_{\mathrm{N}} = \frac{\nu}{\mu + (1 - f)\tau + [1 - (1 - f)b]p\alpha + c}, \tag{6}$$

where c is the cost of preference, induced for example by the energy lost searching for the good type of plant and/or for maintaining the corresponding genetic information. Compared to equation (1) in absence of plant preference, here (eqq. [5] and [6]) probabilities g and f replace the proportion q of toxic plants. If x and $1 - x$ are the proportions of genotypes T and N in the butterfly population, then the mean fitness function of the butterfly population reads

$$\overline{W}(x) = xW_{\mathrm{T}} + (1 - x)W_{\mathrm{N}}.$$

From the slope $\partial\overline{W}/\partial x$ of this mean fitness function, we deduce that the genotype that will ultimately become fixed in the butterfly population is

$$\begin{cases} \mathrm{T} & \text{if } \tau < \alpha pb \\ \mathrm{N} & \text{if } \alpha pb \le \tau \end{cases}. \tag{7}$$

In this equation, the product αp of parasite virulence and prevalence represents the cost of parasitism on the butterfly's fitness. It is the mean-field population-level consequence of the probabilistic individual risk discussed in "Introduction." Genotype T wins over genotype N whenever this cost is above a threshold, the value of which is the ratio τ/b of detrimental and beneficial effects of plant toxicity. Note that this intuitive result does not depend on the proportion q of toxic plants in the environment because of the flexibility of the preference function g: as explained in the previous section, whatever the preference for toxic plants, in absence of toxic plants in the environment, the butterflies will shift to nontoxic plants and this will not affect the birth component of the fitness function. Note also that the threshold of equation (7) does not depend on the cost c since this cost is the same for the two genotypes. Finally, in the particular case where there is no parasite in the environment (i.e., $p = 0$), we can verify that genotype N will invade the population.

Evolution of Genetic Determinism Versus Random Choice

If now y is the proportion of genotypes T and N (i.e., those with a genetically determined plant preference) and $1 - y$ the proportion of genotype R in the butterfly population, we can express the mean fitness function $\overline{W}(y)$ as

$$\overline{W}(y) = \begin{cases} yW_{\mathrm{T}} + (1 - y)W_{\mathrm{R}} & \text{if } \tau < \alpha pb \\ yW_{\mathrm{N}} + (1 - y)W_{\mathrm{R}} & \text{if } \alpha pb \le \tau \end{cases}.$$

This time, from the slope of $\partial\overline{W}/\partial y$, we can predict that the genotype that will ultimately become fixed in the butterfly population is

$$\begin{cases} \mathrm{T} & \text{if } \tau < \alpha pb - \dfrac{c}{g(q) - q} \\ \mathrm{R} & \text{if } \alpha pb - \dfrac{c}{g(q) - q} \le \tau \\ & \qquad \le \alpha pb + \dfrac{c}{g(1 - q) - (1 - q)} \\ \mathrm{N} & \text{if } \alpha pb + \dfrac{c}{g(1 - q) - (1 - q)} < \tau \end{cases}. \tag{8}$$

Recall from equation (3) that all the terms in these inequalities are positive. The condition is here expressed on the plant toxicity τ but can equivalently be put on the parasite virulence α (see app. A). Note from equation (8) that, compared to equation (7), there is now a range of toxicity values τ around αpb (or of parasitism fitness cost αp around the threshold τ/b) that ends up in the fixation of the genotype R that lays eggs at random. The width

$$\begin{aligned} w_\tau &= \frac{c}{g(q) - q} + \frac{c}{g(1 - q) - (1 - q)} \\ &= \frac{c}{(1 - q) - (1 - q)^{\varepsilon}} + \frac{c}{q - q^{\varepsilon}} \end{aligned} \tag{9}$$

of this range linearly increases with the cost c of genetic determinism (fig. 3*A*). From equation (9), we can see that the width of this range also, as expected, increases as the efficiencies $g(q) - q$ and $g(1 - q) - (1 - q)$ of the plant preferences decrease. As shown in figure 2*B*, such a decrease in efficiency happens when the diversity of plants in the environment decreases (minimum reached for $q = 1/2$), and/or when the plant preference ε decreases. Figure 3*B* illustrates the effects of both plant diversity q in the environment and plant preference ε on the width of the band where genotype R wins. In absence of plant preference ($\varepsilon = 1$), we can verify that this width increases to infinity:

$$\lim_{\varepsilon \to 1^+} w_\tau(\varepsilon) = +\infty.$$

Note that here the width of the parametric space where genotype R wins is expressed in units of plant toxicity τ. Exactly the same result is obtained if this width is expressed

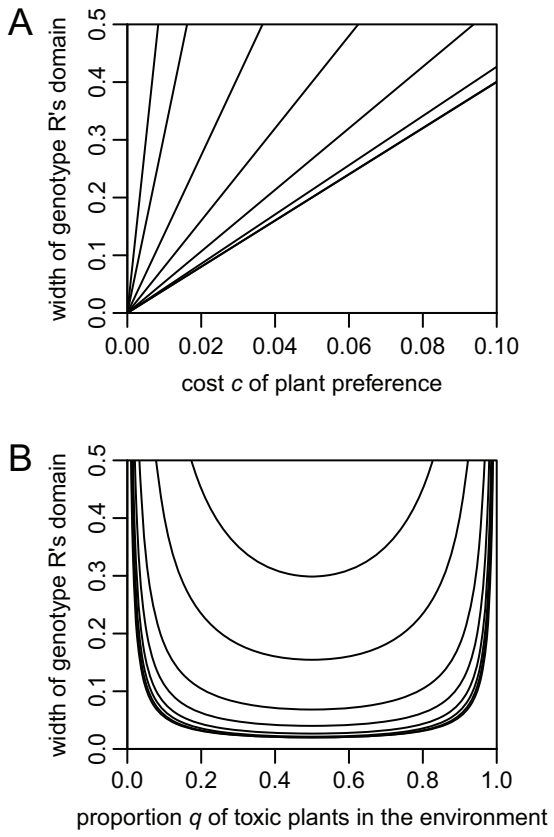

Figure 3: Width of the region of the parametric space (expressed in units of plant toxicity τ; see eq. [9]) where genotype R is fixed, as a function of cost c of plant preference (*A*) and the proportion q of toxic plants in the environment (*B*). The figures are drawn by using equation (2) for the probability g and plant preference ε values are choosen equal to 1.1, 1.2, 1.5, 2, 3, 5, 10, and 100 from top to bottom. The proportion q of toxic plants in the environment is chosen equal to 0.5 for *A*) and the cost c of plant preference is chosen equal to 0.005 for *B*. For illustrative purpose the ranges of the *Y*-axes are limited to [0,0.5] even though the range of the width is naturally [0, $+\infty$].

in units of parasite virulence α (see app. A). Note finally that w_τ does not depend on the parasite prevalence p. This means that, in absence of parasites in the population ($p = 0$), the genotype that will ultimately become fixed in the butterfly population is

$$\begin{cases} \text{R} & \text{if } \tau \le \dfrac{c}{g(1-q)-(1-q)} \\ \text{N} & \text{if } \dfrac{c}{g(1-q)-(1-q)} < \tau \end{cases}.$$

Thus, in absence of parasites in the population, laying eggs preferentially on toxic plants is naturally counterselected because it brings cost (toxicity to the butterfly) without any potential benefit (which would have been toxicity to the parasite). Furthermore, avoiding toxic plants becomes favored by selection as soon as the plant toxicity to the butterfly is above the ratio of cost and efficiency of plant preference.

Modeling Phenotypic Plasticity

We now consider a fourth genotype P for which plant choice is determined by phenotypic plasticity and its fitness function reads

$$W_{\text{P}} = \frac{\nu}{\mu + [pg + (1-p)(1-f)]\tau + (1-gb)p\alpha + c + \varphi} \tag{10}$$

in which the cost τ due to the plant toxicity is equal to its expression in W_{T} (eq. [5]) in proportion p and to its expression in W_{N} (eq. [6]) in proportion $1 - p$. This conveys the fact that the plant type the butterfly lays its eggs on depends on its infection status (p vs. $1 - p$ at the population level). As for the cost α due to parasite virulence, the butterfly cannot change the probability p of being infected, but if infected, it will choose to lay eggs on the toxic plants, thus reducing the cost of parasite virulence by the factor $1 - gb$. Finally, compared with fitness expressions W_{T} and W_{N}, W_{P} contains an additional cost φ of phenotype plasticity that accounts for the energy used to assess the infection status and take a decision in consequence.

Phenotypic Plasticity Versus Genetic Determinism

Applying the same rationale as above, if y is now the proportion of genotype P and $1 - y$ the proportion of genotypes N and T, the mean fitness function $\overline{W}(y)$ reads

$$\overline{W}(y) = \begin{cases} yW_{\text{P}} + (1-y)W_{\text{T}} & \text{if } \tau < \alpha pb - \dfrac{c}{g(q)-q} \\ yW_{\text{P}} + (1-y)W_{\text{N}} & \text{if } \alpha pb + \dfrac{c}{g(1-q)-(1-q)} < \tau \end{cases},$$

and, again from the slope of $\partial\overline{W}/\partial y$, we can predict that genotype P will win over genotype T if and only if

$$\tau > \frac{\varphi}{(1-p)(g(q)+g(1-q)-1)} \text{ and } \alpha > \frac{\tau}{pb} + \frac{c}{(g(q)-q)pb}. \tag{11}$$

This means that there is a threshold on the plant toxicity τ above which phenotypic plasticity will replace genetically determined preference for toxic plants. As expected, this threshold increases with the cost φ of phenotypic plasticity: the higher the cost of phenotypic plasticity, the higher the

plant toxicity has to be for a shift from genetically determined toxic plant preference to phenotypic plasticity. This threshold also increases with the parasite prevalence p: the higher the parasite prevalence, the higher level of plant toxicity genotype T can stand. In the extreme case where the parasite prevalence is equal to 1,

$$\lim_{p\to 1^-} \frac{\varphi}{(1-p)[g(q)+g(1-q)-1]} = +\infty,$$

meaning, from equation (11), that genotype P never wins over genotype T. Recall from the previous section that, in absence of the parasite ($p = 0$), genotype T never wins. Finally, this threshold decreases as the proportion q of toxic plants in the environment departs from 50% meaning, as expected, that the benefit of phenotypic plasticity increases with environmental diversity. Similarly, we can show that genotype P wins over genotype N when

$$\tau > pb\alpha + \frac{c}{g(1-q)-(1-q)} \text{ and } \alpha > \frac{\tau}{b} + \frac{\varphi}{[g(q)+g(1-q)-1]bp}. \quad (12)$$

Note from equations (3) and (4) that the thresholds of equations (11) and (12) are all positive.

Phenotypic Plasticity Versus Random Choice

From equation (8) we know that genotype R wins over any genetically determined plant choice genotype (T and R) whenever

$$\alpha pb - \frac{c}{g(q)-q} \le \tau \le \alpha pb + \frac{c}{g(1-q)-(1-q)}. \quad (13)$$

Under these conditions, if y is now the proportion of genotype P and $1 - y$ the proportion of genotype R, the mean fitness function reads

$$\overline{W}(y) = yW_{\mathrm{P}} + (1-y)W_{\mathrm{R}},$$

from which we can deduce that genotype P wins over genotype R whenever the conditions of equation (13) and

$$\alpha > \frac{[(1-p)(1-g(1-q)) + pg(q) - q]\tau + c + \varphi}{(g(q)-q)pb}$$

are fulfilled. Note that the α-intercept of this condition is always positive but that the sign of its slope can be either positive of negative, depending on the parasite prevalence p and the proportion q of toxic plants in the environment.

Modeling the Evolution of Medication

Figure 4 summarizes the results of the previous sections and shows that a condition for phenotypic plasticity to evolve is high plant toxicity τ and high parasite virulence α. For low plant toxicity and low parasite virulence, absence of preference is the optimal evolutionary strategy. Finally, genetically determined plant choice will win when either of plant toxicity or parasite virulence is high. Under these conditions, phenotypic plasticity thus appears to be the best evolutionary strategy to deal with two contradictory selective pressures. It can easily be proven that the lines that delineate domains where each of genotypes N, P, and R wins intersect in one single point and that the lines that delineate domains where genotypes T, P, and R wins intersect in one single point too. See appendix B for equations of these five lines and coordinates of the two intersection points. q6

We can verify from figure 4 that the absence of plant diversity in the environment (i.e., $q = 0$ or $q = 1$) is a sufficient condition for genotype R to invade the butterfly population. We can also verify from this figure that a condition for phenotypic plasticity to emerge is that parasite prevalence p has to be different from 0 and 1. Figure 5 shows that in absence of parasites ($p = 0$), the genotype of the butterfly depends solely on the plant toxicity τ, with genotype N winning as soon as the plant toxicity becomes too high:

$$\tau > \frac{c}{g(1-q)-(1-q)},$$

and genotype R winning otherwise. Note that this threshold expectedly increases with the cost of plant choice and with the proportion $1 - q$ of nontoxic plants in the environment. Figure 5 also shows that, when the parasite prevalence $p = 1$, genotype N wins for high plant toxicity

$$\tau > \frac{c}{g(1-q)-(1-q)} + b\alpha,$$

genotype T wins for high parasite virulence

$$\alpha > \frac{c}{(g(q)-q)b} + \frac{\tau}{b},$$

and genotype R wins otherwise.

Plant Toxicity and Medicinal Properties

We have considered so far that plant toxicity τ and the relative decrease b in virulence due to plant toxicity are two independent parameters. However, it is likely that the detrimental effect of plant toxicity on the butterfly and its parasite are correlated. The minimum is to consider that

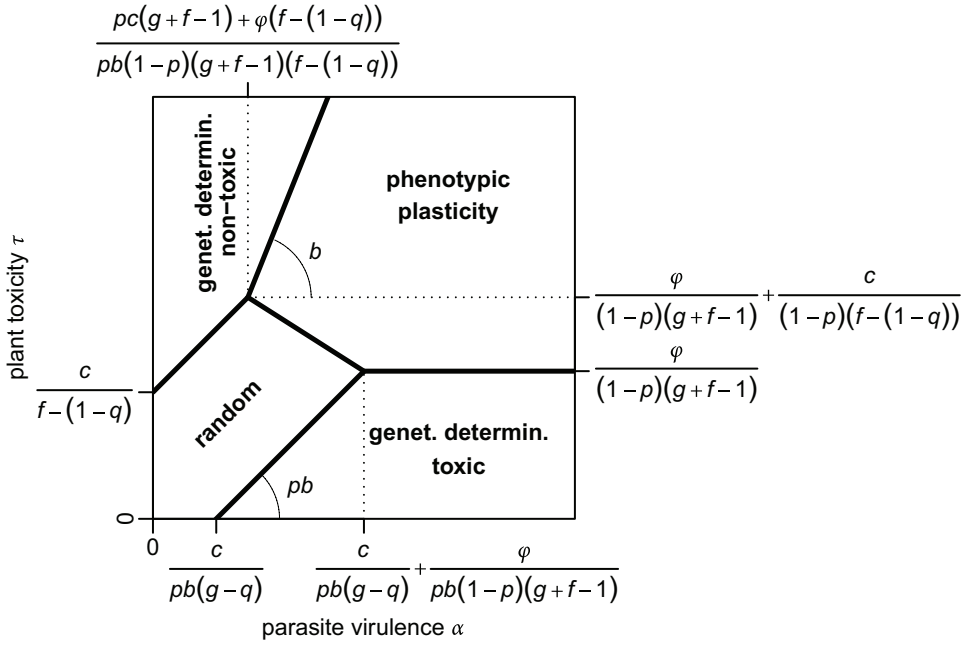

Figure 4: Evolution of plant choice determinism of a butterfly population as a function of parasite virulence α and plant toxicity τ. Parameter p is the parasite prevalence, and q is the proportion of toxic plants in the environment. Plant preference is modeled by $g = g(q)$ and $f = g(1 - q)$, see "Model Development: Plant Preference." Parameters c and φ are the cost of plant preference and phenotypic plasticity, respectively. Butterfly genotypes are R (no plant preference, random choice), T and N (genetically determined preference of toxic and nontoxic plants, respectively), and P (phenotypic plasticity). Circle arcs materialize slopes of $\tau(\alpha)$ functions.

$$\lim_{\tau \to 0^+} b(\tau) = 0^+ \text{ and}$$

$$\lim_{\tau \to +\infty} b(\tau) = 1^-.$$

Such a relationship can phenomenologically be modeled by a cumulative gamma distribution:

$$b(\tau) = \frac{\gamma\left(k, \frac{\tau}{\theta}\right)}{\Gamma(k)}, \tag{14}$$

with shape and scale parameters k and θ respectively where Γ is the gamma function and γ is the lower incomplete gamma function:

$$\Gamma(k) = \int_0^{+\infty} x^{k-1} e^{-x} dx \text{ and}$$

$$\gamma\left(k, \frac{\tau}{\theta}\right) = \int_0^{\tau/\theta} x^{k-1} e^{-x} dx.$$

The cumulative gamma distribution has the required property of being a continuously increasing function defined from R^+ (the domain of definition of parameter τ) to [0,1] (the domain of definition of parameter b). Its exact shape is controlled by two parameters: θ, the scale parameter, controls the initial relationship, from linear to exponential, and k, the shape parameter, controls how fast the asymptote is reached. See figure C1 in appendix C for illustrations of the effects of these two parameters on the relationship between virulence attenuation b and plant toxicity τ. Replacing parameter b by the function $b(\tau)$ of equation (14) in order to account for such a relationship between of effects of plant toxicity on the parasite (b) and on the butterfly (τ) brings only one qualitative change to the general pattern of figure 4: genotype R will always win as long as the plant toxicity τ is low enough (see fig. 6*A*, 6*B*). Conclusions regarding the conditions where phenotypic plasticity gets fixed are unchanged (see fig. 6*A*, 6*C*). See figure C1 in appendix C for the effects of shape and scale parameters k and θ on the pattern of figure 6.

Discussion

Our game-theory model allows us to investigate the determinism (none, genetically fixed, or phenotypically plastic) of plant choice (toxic vs. nontoxic) when plant toxicity is detrimental not only to the parasites but also to the butterfly. The model explicitly takes into account not only the parasite prevalence and virulence but also the pro-

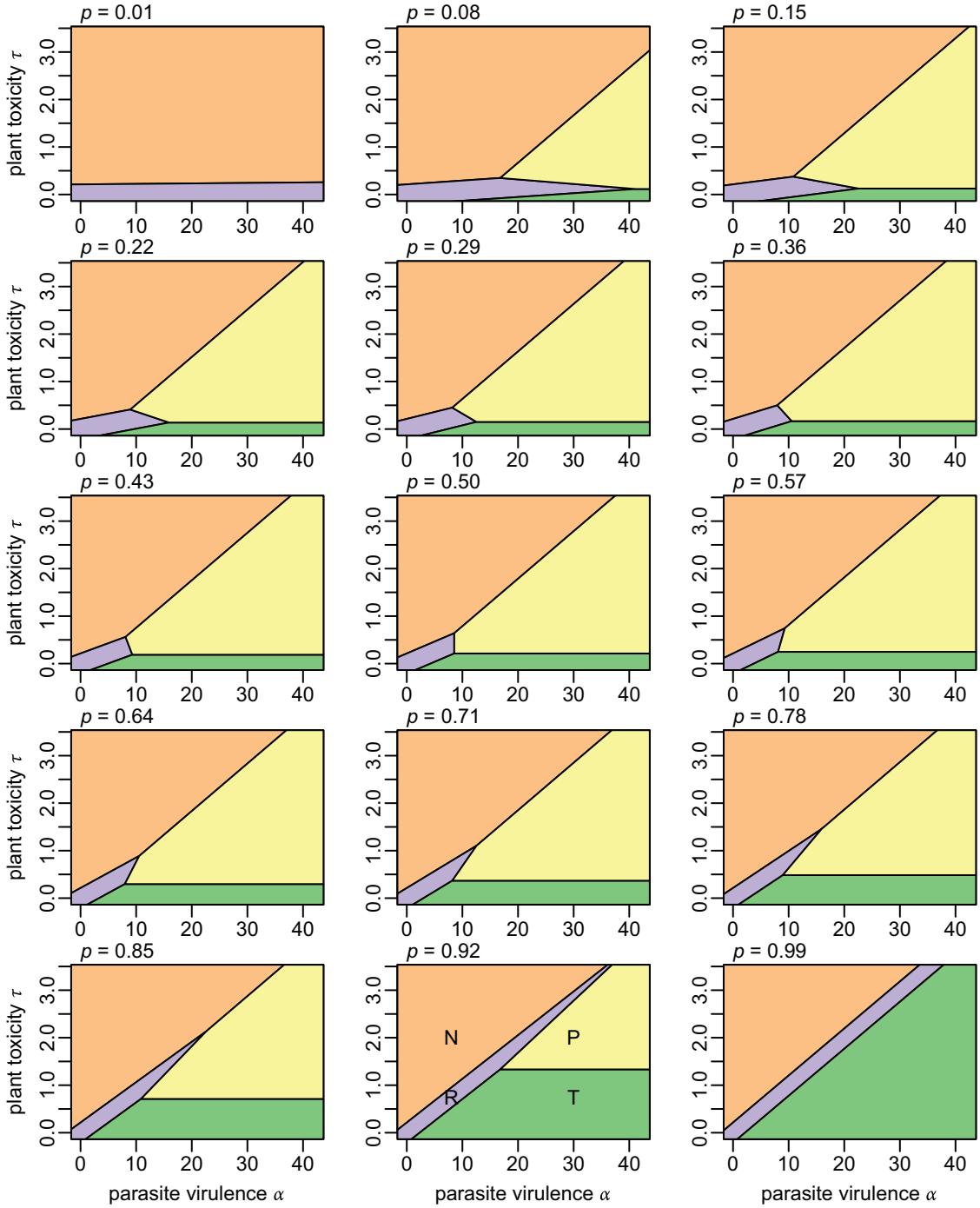

Figure 5: Effect of parasite virulence α and plant toxicity τ on the evolution of medication, for different values n parasite prevalence p. Parasite prevalence varies from 0.01 to 0.99 from left to right and from top to bottom. Other parameter values are $q = 0.5$, $c = 0.1$, $\varphi = 0.1$, $b = 0.1$, and $\varepsilon = 5$. Note the slope of the domain where genotype R wins: it increases from 0 to b as p increases from 0 to 1. The intersections between domains N, P, R and domains T, P, R tends toward infinity when p tends toward 0 or 1 (see eqq. in fig. 4 and appendix B). See also http://marcchoisy.free.fr/medication/ for an animated version of this figure when parasite prevalence p increases from 0 to 1.

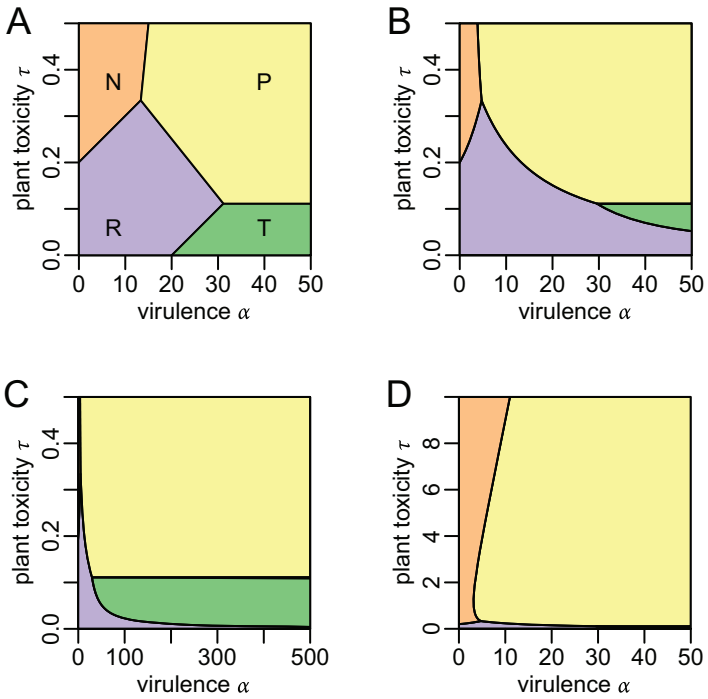

Figure 6: Evolution of plant choice determinism of a butterfly population as a function of parasite virulence α and plant toxicity τ when virulence attenuation b is independent of plant toxicity τ (*A*) and when it is linked to plant toxicity τ according to equation 14 (*B–D*). Compared to *B*, *C* shows a wider range of virulence, highlighting the fixation of genotype R, even for high parasite virulence, as long as the plant toxicity is low enough. *D* shows a wider range of plant toxicity, showing that, as in figures 4 and 5, genotype N gets fixed when plant toxicity gets too high. Parameter values are $q = 0.5$, $p = 0.1$, $c = 0.1$, $\varphi = 0.1$ and equation (2) is used to model the preference, with $\varepsilon = 10$. Color code is the same as in figure 5.

portion of toxic plants in the environment since the plant actually chosen by the butterfly depends not only on the strength of preference but also on its availability in the environment. The determinism of plant choice that is eventually favored by selection depends on (i) the availability of toxic plants in the environment and their relative toxicities to the butterflies and their parasites, and (ii) on the parasite prevalence and virulence.

Our model reveals some intuitive patterns. First, when there are no parasites in the population (prevalence $p = 0$), butterflies that are genetically determined to use toxic plants (genotype T) and butterflies that plastically alter their preference based on infection (genotype P) do not persist; these results are easily explained on the basis of the costs associated with using toxic plants and the costs of plasticity itself. Instead, butterflies that oviposit at random (genotype R) are selected for below a threshold level of plant toxicity, and butterflies that are genetically determined to preferentially oviposit on nontoxic plants (genotype N) are selected for above that threshold level. On the other extreme, when parasite prevalence reaches 100%, butterflies that are genetically determined to use toxic plants (genotype T) are selected for, especially when parasite virulence is high.

The most interesting results of the model occur when parasite prevalence lies between 0% and 100%. Butterflies that medicate plastically are not selected for until parasite prevalence reaches a threshold (see fig. 5). With increasing parasite prevalence, these plastic butterflies start being selected for lower parasite prevalence. This is the case up to 50% prevalence. For parasite prevalences higher than 50%, the parameter domain in which plastic butterflies are selected for becomes smaller, and phenotype plasticity starts being selected for higher plant toxicities. When parasite prevalence reaches high levels, selection no longer favors plastic butterflies. Instead, butterflies that are genetically fixed to use toxic plants are selected for. Overall, then, this mathematical model largely supports the verbal model on genetically fixed and phenotypically plastic forms of medication that has been suggested in "Introduction."

One main strength of our mathematical model is that it explicitly accounts for the availability of plants of different types (toxic vs. nontoxic) in the environment. As a first theoretical exploration, the analysis of our model is

here voluntarily based on comparisons of the fitnesses of only two genotypes (out of four) at a time, and it shows no polymorphism. Our model also considers the simple case where parasite prevalence p is a fixed parameter, thus preventing any epidemiological feedback on the evolution of medication (where medication affects parasite prevalence which, in return, affects the evolution of medication). Density dependence through such epidemiological feedback and the consideration of more than two genotypes at a time could maintain polymorphism. These two limitations (no more than two genotypes at a time and absence of epidemiological feedback) allowed us to analytically dissect the mechanisms of the evolution of medication, leading to simple predictions. An adaptive-dynamic version of our model, relaxing these limitations, will be developed and analyzed in a subsequent article. Further models could be developed on the basis of coevolution of host and parasite. For example, it is possible that the use of medicinal plants selects for more virulent parasites (de Roode et al. 2011*a*), which in turn increases selection for medication.

Based on our simple game-theory model, it is expected that monarchs have evolved plastic medication in areas such as the eastern and western United States, where parasite risk is low. In contrast, our model predicts that monarchs evolve genetically fixed medication in places like south Florida, where parasite population is high. So far, studies have been done only with monarchs from eastern (Lefèvre et al. 2012) and western United States (Lefèvre et al. 2010), and monarchs from both of these areas indeed medicate plastically. However, there are no data yet to determine whether monarchs from high-risk areas have evolved genetically fixed medication.

In general, our model may serve as a useful framework to make predictions about different types of medication in different systems. For example, wood ants incorporate antimicrobial resin in their nests (Christe et al. 2003; Chapuisat et al. 2007; Castella et al. 2008). This is a genetically fixed medication behavior, and our model suggests that the costs of this behavior are low, which could indeed be the case if the collection of resin bears few costs in terms of reducing the collection of structural and food items.

As mentioned above, further developments of our model will include not only frequency dependence (as it currently does through the effect of the prevalence parameter p on the evolution of medication), but also density dependence (in order to account for a negative epidemiological feedback of the evolution of medication on the parasite prevalence). Furthermore, our model assumes that the monarch butterflies either have a genetically fixed form of medication or a plastic strategy that is entirely based on changing the behavior in response to parasite infection. However, it has often been suggested that medication may be based on associative and social learning (reviewed and criticized in Hart 2005). Such a social transmission of medication can also be added to our model, in a way similar to the way the transmission of the disease is modeled. As for the latter, it will thus add frequency and potentially density dependence. Interestingly however, contrary to the disease transmission process, the density-dependent feedback will be positive instead of negative.

Overall, our model is the first attempt at a quantitative understanding of the evolution of animal medication. Although we have made some strong assumptions that will be relaxed in future models, the simplicity of our model allows us to make clear predictions that can be tested in natural systems. As recent studies suggest, animal medication is highly prevalent in wide range of insects (reviewed in de Roode et al. 2013). Since insects can often be easily used for manipulative experiments, and because selection experiments are possible with the fast-lived animals, we suggest that insect systems may be the most suitable systems to test model predictions and advance our understanding of the evolution of animal medication.

APPENDIX A

Genetic Determinism Versus Random Choice

The conditions of equation (8) on plant toxicity τ:

$$\begin{cases} \text{T} & \text{if } \tau < \alpha pb - \dfrac{c}{g(q) - q} \\ \text{R} & \text{if } \alpha pb - \dfrac{c}{g(q) - q} \le \tau \le \alpha pb + \dfrac{c}{g(1-q) - (1-q)} \\ \text{N} & \text{if } \alpha pb + \dfrac{c}{g(1-q) - (1-q)} < \tau \end{cases}$$

can be reexpressed by putting conditions on parasite virulence α where the genotype that gets fixed is

$$\begin{cases} \text{N} & \text{if } \alpha < \dfrac{\tau}{pb} - \dfrac{c}{[g(1-q) - (1-q)]pb} \\ \text{R} & \text{if } \dfrac{\tau}{pb} - \dfrac{c}{[g(1-q) - (1-q)]pb} < \alpha < \dfrac{\tau}{pb} + \dfrac{c}{[g(q) - q]pb} \\ \text{T} & \text{if } \dfrac{\tau}{pb} + \dfrac{c}{[g(q) - q]pb} < \alpha \end{cases}$$

or on conditions on the fitness cost αp of parasitism, with the genotype that gets fixed being:

$$\begin{cases} \text{N} & \text{if } \alpha p < \dfrac{\tau}{b} - \dfrac{c}{[g(1-q)-(1-q)]b} \\ \text{R} & \text{if } \dfrac{\tau}{b} - \dfrac{c}{[g(1-q)-(1-q)]b} < \alpha p < \dfrac{\tau}{b} + \dfrac{c}{[g(q)-q]b} \\ \text{T} & \text{if } \dfrac{\tau}{b} + \dfrac{c}{[g(q)-q]b} < \alpha p. \end{cases}$$

Another expression, using only strictly positive thresholds, is to state that genotype T is fixed whenever

$$\alpha > \frac{\tau}{pb} + \frac{c}{(g(q)-q)pb},$$

and genotype N is fixed whenever

$$\tau > \alpha p b + \frac{c}{g(1-q)-(1-q)},$$

and genotype R is fixed otherwise. The width of the range of the parametric space where genotype R is fixed, expressed in units of the plant toxicity τ in equation (9):

$$\begin{aligned} w_\tau &= \frac{c}{g(q)-q} + \frac{c}{g(1-q)-(1-q)} \\ &= \frac{c}{(1-q)-(1-q)^\varepsilon} + \frac{c}{q-q^\varepsilon} \end{aligned}$$

can alternatively be expressed in units of parasite virulence α:

$$\begin{aligned} w_\alpha &= \left[\frac{1}{g(q)-q} + \frac{1}{g(1-q)-(1-q)}\right] \cdot \frac{c}{bp} \\ &= \left[\frac{1}{(1-q)-(1-q)^\varepsilon} + \frac{1}{q-q^\varepsilon}\right] \cdot \frac{c}{bp}. \end{aligned}$$

The conclusions drawn on w_α are the same as the ones drawn in the main text on w_τ except that the expression of w_α is also affected by the medicinal property b of plant toxicity. We can verify that

$$\lim_{p\to 0} w_\alpha(p) = +\infty,$$

which is consistent with the results on w_τ derived in the main text.

APPENDIX B

Domain Limits and Intersections

Table B1: Equations of the lines delineating the domains of the $\alpha - \tau$ parametric space where genotypes R, T, N, and P wins, expressed either as functions of τ or as functions of α

Line or point	α	τ
R-N	$\alpha(\tau) = \frac{[f-(1-q)]\tau - c}{[f-(1-q)]bp}$	$\tau(\alpha) = \frac{c}{f-(1-q)} + bp\alpha$
R-T	$\alpha(\tau) = \frac{c}{(g-q)bp} + \frac{\tau}{bp}$	$\tau(\alpha) = bp\alpha - \frac{c}{g-q}$
P-N	$\alpha(\tau) = \frac{\varphi}{(f+g-1)bp} + \frac{\tau}{b}$	$\tau(\alpha) = b\alpha - \frac{\varphi}{(f+g-1)p}$
P-T	—	$\tau(\alpha) = \frac{\varphi}{(1-p)(f+g-1)}$
P-R	$\alpha(\tau) = \frac{c+\varphi-[f-(1-q)-(g+f-1)p]\tau}{(g-q)bp}$	$\tau(\alpha) = \frac{c+\varphi-(g-q)bp\alpha}{f-(1-q)-(g+f-1)p}$
N-P-R	$\alpha = \frac{(g+f-1)cp+[f-(1-q)]\varphi}{(g+f-1)[f-(1-q)](1-p)bp}$	$\tau = \frac{c}{(1-p)[f-(1-q)]} + \frac{\varphi}{(1-p)(g+f-1)}$
T-P-R	$\alpha = \frac{c}{(g-q)bp} + \frac{\varphi}{(g+f-1)(1-p)bp}$	$\tau = \frac{\varphi}{(1-p)(f+g-1)}$

Note: The last two lines show the coordinates of the intersection points between domains N, P, R and T, P, R, respectively. These equations are used to draw figures 4 and 5. Recall that $g = g(q)$ and $f = g(1 - q)$ where q is the proportion of toxic plants in the environment. See main text for meaning of other parameters.

APPENDIX C

Relationship between b and τ

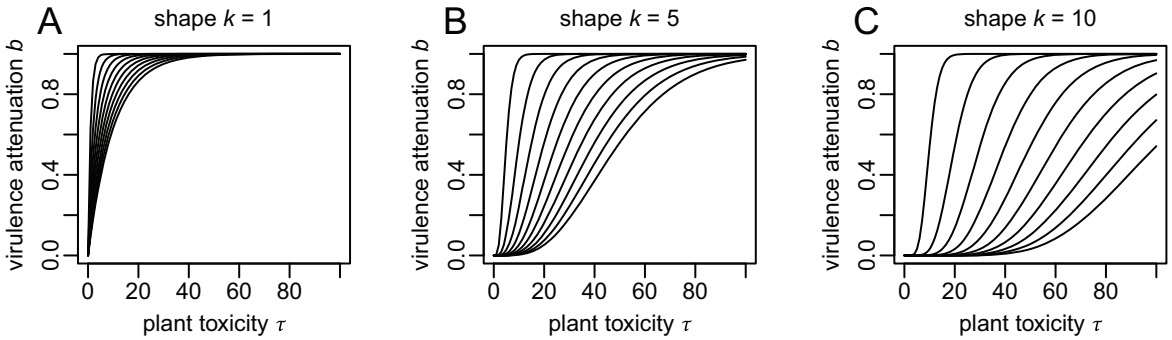

Figure C1: Relationship between virulence attenuation b due to plant toxicity and the detrimental effect τ of plant toxicity on butterfly fitness. In this example the relationship follows a cumulative gamma distribution of shape and scale parameters k and θ, respectively (see eq. [14]). Shape $k = 1$, 5 and 10 in *A*, *B*, and *C*, respectively. In each panel, scale θ varies from 1 to 10, by step of 1 left to right.

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