

Dynamics of Production of Sexual Forms in Aphids: Theoretical and Experimental Evidence for Adaptive “Coin-Flipping” Plasticity

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ABSTRACT: The best strategy for an organism to deal with unpredictable environmental conditions is a stochastic one, but it is not easy to distinguish it from nonadaptive randomness in phenotype production, and its convincing demonstrations are lacking. Here we describe a new method for detection of adaptive stochastic polyphenism and apply it to the following problem. In fall, each female of the bird cherry–oat aphid, *Rhopalosiphum padi*, faces a decision either to produce sexuals, which mate and lay cold-tolerant eggs, or to continue production of cold-sensitive parthenogenetic females,

which potentially yields a higher population growth rate but is risky because a cold winter can kill all of her descendants. Using a simulation model, we show that global investment in sexual reproduction should be proportional to winter severity and that variance in the peak date of production of sexual individuals should depend on climate predictability. Both predictions are validated against standardized trap data on aphid flight accompanied by meteorological data, and the predictions support adaptive phenotypic plasticity.

Keywords: *Rhopalosiphum padi*, stochastic polyphenism, bet hedging, risk spreading, evolution of plasticity, overwintering strategy.

Phenotypic plasticity, that is, the ability of a genotype to develop different phenotypes in different environments (Stearns 1989), is an important characteristic that is subject to natural selection. Evolution, however, often acts under stochastic conditions and the consequences of stochasticity on evolution of phenotypic plasticity are poorly understood (Orzack 1993).

Phenotypic plasticity in insects has usually been equated with predictive plasticity, or conditional polyphenism (Walker 1986), in which a genotype responds to different current environments by producing different phenotypes in a way that maximizes its fitness. Here we will thus use the term “conditional polyphenism” to describe this form of plasticity corresponding, for a single genotype, to a response to a given current environment by deterministically producing a given phenotype. However, the “decision” made now will generally have consequences on future fitness although the future state of the environment cannot be perfectly predicted on the basis of the current one. Thus there is a delay between the instant when the decision is made and the instant when it affects individual fitness, and during this delay the environment may change (Moran 1992). In such a case, a stochastic decision, called adaptive coin-flipping (Cooper and Kaplan 1982; Kaplan and Cooper 1984) or stochastic polyphenism (Walker 1986), can be fitter (Cooper and Kaplan 1982; Haccou and Iwasa 1995; Menu et al. 2000) and can lead to diversified

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bet hedging (Seeger and Brockman 1987; Hopper 1999; Menu et al. 2000; Menu and Desouhant 2002). Here we will thus use the term “stochastic polyphenism” to describe the form of plasticity corresponding to a response by a single genotype to a given current environment by stochastically (e.g., flipping a coin) producing one phenotype among a set of possible phenotypes.

Because an organism can never perceive its environment with complete accuracy, all decision making is made under some uncertainty, and this frequently leads to a selective advantage for genotypes performing stochastic polyphenism. This form of plasticity, which copes with uncertainty, can thus be expected to be widespread in nature (Walker 1986; see also Moran 1992 for a discussion).

Two sets of genetic, possibly distinct, factors are involved in expression of polyphenism: structural genes determine the building of a given form and regulatory genes are responsible for the switch between the different forms. Phenotypic variability of polyphenism results from a differential expression of those genes within a given genotype. While conditional polyphenism would result from deterministic control by means of regulatory genes, which are sensitive to some environmental threshold (physiological epistasy), stochastic polyphenism would result instead from stochastic interactions between regulatory genes (which are sensitive to environmental variations), thus leading to statistical epistasy (Pigliucci 1996). In other words, under stochastic polyphenism, each individual “flips a coin” and adopts one of the possible tactics with a certain probability (Cooper and Kaplan 1982).

Surprisingly, empirical examples of the existence of stochastic polyphenism as an adaptive strategy are lacking, possibly because it is difficult to distinguish it from other nonadaptive alternative explanations (Stearns 1989). Here we describe a method for identification of stochastic polyphenism from empirical data and apply it to a specific example. The central idea is this: it is impossible to judge from a single observation whether phenotypic variation is adaptive or just random. However, if phenotypic variation is adaptive, then it is positively correlated with environmental uncertainty, which can be tested against data from different environments.

Aphids reproduce parthenogenetically for at least part of the year, which results in lack of recombination (Blackman 1979). Thus, barring mutation, all descendants of a parthenogenetic female are genetically identical and any variation among them (winged or wingless forms; sexual or parthenogenetic forms) results from phenotypic plasticity. This makes them remarkably suitable to the study of polyphenism (Walker 1986). The clone is the natural selection unit in aphids (Austin et al. 1996). Therefore, each individual is playing a cooperative game, and fitness

of the clone, not that of an individual, is maximized (Hutchinson 1996).

The purpose of this article is to test whether phenotypic variation in the production of sexual forms corresponds to adaptive coin-flipping plasticity in the bird cherry–oat aphid in natural populations of *Rhopalosiphum padi* or whether it is just a nonadaptive fluctuation. We test this by comparing the predictions of a simulation model to empirical data.

In our model, two sources of uncertainty were considered. The first source of uncertainty is related to the probability of the winter being harsh. This harshness determines the winter survival of the mobile forms but does not affect the eggs resulting from the sexual reproduction. The second source of uncertainty is associated with the timing of arrival of winter. This variable determines the relative fitness value associated with any possible date of switching from the parthenogenetic to the sexual mode of reproduction.

The first source of uncertainty and its evolutionary consequences has been widely studied elsewhere (Rispe and Pierre 1998; Rispe et al. 1998a, 1998b). We therefore focused mainly on the second aspect. Consequently, the main question we addressed was, is the observed variability in the switching time from the parthenogenetic to the sexual mode of reproduction adaptive (stochastic polyphenism hypothesis), or does it result from random fluctuations around a deterministic fixed switching date (conditional polyphenism hypothesis)? In aphids, the environmental cue for the switch from parthenogenetic to sexual mode of reproduction is mainly photoperiod, modified to some extent by temperature (MacGillivray and Anderson 1964; Blackman 1971; Dixon and Glen 1971; Tsitsipis and Mittler 1976, 1977a, 1977b; Wegorek and Dedryven 1987). A conditional polyphenism strategy would then consist of deterministic switches from parthenogenetic to sexual reproduction when some specific conditions of day length and temperature are met. An optimal strategy is then to switch to the production of sexual forms at a time when the expected fitness is maximized. In this deterministic case, no adaptive variation in the time when sexual forms are produced should be observed. Nonadaptive variation can, however, appear for three main reasons: genetic variation such that individual behavior does not perfectly match the optimal strategy, small-scale variability in the environment of individuals, and variability in the perception of the same environment by different individuals. All these sources of variability would result in some nonadaptive, random variation in the time of production of sexuals around the optimal timing. Conversely, under stochastic polyphenism, adaptive variation is expected to have evolved to precisely match environmental uncertainty. The aim of our work was to

distinguish between these two sources of variation (random vs. adaptive). Our approach was based on the following idea: under the conditional polyphenism assumption, no relationship between the variance in the time of production of sexuals and the environmental uncertainty (expressed as the variance in the timing of arrival of winter) is expected, whereas under the assumption of stochastic polyphenism such a variance-to-variance relationship is expected.

A related question was addressed earlier by Ward et al. (1984), who suggested an interesting model predicting the precise time of the switch to production of sexual forms based on the temperatures experienced. They predicted a positive correlation between the mean peak of male production and the mean date of arrival of winter. However, in their model, the stochastic aspect and the analysis of its adaptive importance, which is now included in our work, were lacking.

Material and Methods

Biological Background

In temperate climates, many species of aphids are holocyclic, that is, they have several parthenogenetic generations during spring and summer and one sexual generation in fall that produces eggs (Hullé et al. 1999; Simon et al. 2002). Diapausing eggs are generally considered to be cold resistant, which enables them to survive winter (Sømme 1969; Leather 1992; Rispe et al. 1998b). If winter is mild, then parthenogenetic females may also survive and even complete several generations (Leather 1992; Rispe and Pierre 1998; Simon et al. 2002). This may account for the persistence of anholocyclic aphid clones that reproduce parthenogenetically all year, which is common in areas where the winter is mild (Pons et al. 1992, 1995; Zhou et al. 1995; Simon et al. 1996; Rispe et al. 1998a; Delmotte et al. 2001). There is a trade-off between holocyclic and anholocyclic ways of life: production of parthenogenetic individuals means a larger population growth rate in favorable environmental conditions, but adverse climatic conditions can result in extinction of the whole anholocyclic clone while the diapausing eggs from holocyclic lines can survive (Leather 1992).

In the bird cherry–oat aphid, *Rhopalosiphum padi*, sexual reproduction is associated with host alternation from various herbaceous plants (secondary hosts) to their primary host, the bird cherry, *Prunus padus* (fig. 1). In fall, holocyclic parthenogenetic females give birth to males and a special type of parthenogenetic females called gynoparae. Males and gynoparae fly to a bird cherry where the latter give birth to sexual females, oviparae, which after maturation mate with males and lay eggs (Leather et al. 1989;

Austin et al. 1996; Lushai et al. 1996). Anholocyclic parthenogenetic females always produce parthenogenetic females and sometimes also some males (Simon et al. 1991). Persistence of anholocyclic clones after a harsh winter is enabled either via descendants of these few males (Rispe and Pierre 1998; Rispe et al. 1998b) or via long-distance immigration from other areas with milder climates.

Leaf fall of the bird cherry is the deadline for production and development of oviparae (Glinwood and Pettersson 2000). The combination of the deadline and the delay (the two generations that must be completed between allocation to gynoparae and oviposition by oviparae; fig. 1) determines the time when a parthenogenetic female living on the summer host has to produce the sexual forms migrating to the winter host (Ward and Wellings 1994). She has to estimate whether there is still enough time to produce ordinary parthenogenetic offspring or whether winter is so close that it is necessary to produce dispersing gynoparae or males (Ward et al. 1984). If the date of arrival of winter was perfectly predictable, an abrupt switch from asexual to sexual reproduction (except for some random nonadaptive fluctuations) would be selected.

In the following section, we present a model predicting the sequence of sexual form production during fall and winter, which maximizes fitness of the genotype. Severity and predictability of climate are used as model parameters. The model predicts that when the date of arrival of winter is not perfectly predictable, then the best strategy is to invest in both forms at any instant, with the rate of investment in the sexual form being positively correlated with the instantaneous probability of occurrence of the date of arrival of winter during the next two generations. Model predictions are then tested against biological data: suction trap catches that describe the dynamics of flight and timing of reproduction of *R. padi* in various European sites and the meteorological data related to those locations.

The Model

We studied the behavior of a dynamic stochastic variable—the timing of production of sexual forms—as a function of a temporal sequence of decisions. At each of a certain number of instants that represented individual choices, the probability of choosing a given form was determined. One time step was assumed to be equal to one generation, and thus a newly born parthenogenetic individual matured in one step. The sequence of decisions (or overwintering strategy) is illustrated in figure 2. Table 1 sums up the parameters used in the model.

We assumed that at each site there exists a certain date, T_{end} ($< T_{\text{max}}$; the date of arrival of winter), after which no more eggs can be laid. This date may be understood as, for example, the date of leaf fall of the bird cherry tree,

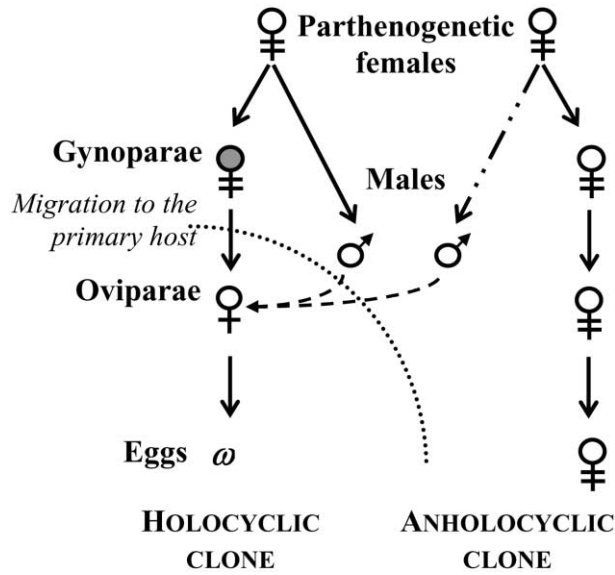


Figure 1: Scheme of sexual morph production in autumn in the aphid species *Rhopalosiphum padi* (adapted from Rispe et al. 1998b). Holocyclic clones invest all reproductive resources in sexual reproduction, producing both males and sexual females (gynoparae + oviparae). Anholocyclic clones reproduce only parthenogenetically and occasionally produce some males (androcyclic clones).

after which no more sexual individuals are able to develop and reproduce. This date is not the same each year and is characterized only by its probability distribution. We assumed a normal distribution with the mean μ ($= T_{\max}/2$ in our simulations) and variance σ^2 . At each of a finite number of instants ($T_1, T_2, \dots, T_{\max}$), a parthenogenetic female (on herbaceous plants) was assumed to decide between two possibilities.

The first possibility is to produce sexual individuals that, provided they mature before the date of arrival of winter, produce a total of $\lambda > 1$ eggs, which are cold tolerant and therefore able to survive until hatching the following spring. This corresponds to the “saver” strategy (Rispe et al. 1998b), and we assumed that a female chooses this option with probability β_i .

The second possibility is to produce $\lambda > 1$ parthenogenetic forms, which will face the same decision again when they mature. This strategy can potentially yield a higher growth rate but is risky because it delays production of sexuals. Indeed, sexual individuals produced after T_{end} (the date of arrival of winter) will fail to reproduce, and no eggs will be laid. This corresponds to the “gambler” strategy (Rispe et al. 1998b), and we assumed that a female chooses this option with probability $1 - \beta_i$. At first glance, there is no reason for λ to be the same in sexual individuals and in parthenogens. However, in our model, the “cost of

sex” is determined by λ to the power of the number of generations that parthenogens can achieve during winter after sexuals have laid eggs. Accordingly, the cost of sex increases with λ , and any cost value can be simulated by using the appropriate value of λ . Thus for the sake of simplicity, λ was assumed to be identical in both forms. We assumed that once a female starts production of sexual forms, her parthenogenetic reproduction is over for the season.

The date T_{\max} is defined as the arrival of spring, when all eggs will hatch. At this date the simulation was stopped, and the total number of descendants was counted. We assumed that individuals are not able to collect any information on the arrival of winter, so individuals who are scheduled to reproduce sexually continue to do so even after T_{end} . All the eggs produced before T_{end} were assumed to survive winter while sexual individuals produced after T_{end} were considered to fail to reproduce. In addition, the clone can continue to reproduce partly parthenogenetically until T_{\max} when the winter is mild (occurring with probability $1 - p$). It was set $T_{\max} = 50$, which corresponds to half-week intervals between two consecutive dates T_i between mid-August and mid-February.

The overwintering strategy was defined by a vector of $\beta = \{\beta_i\}_{i \in (1, \dots, \max=50)}$ describing the proportion of sexual forms produced by the parthenogenetic individuals at each of the consecutive dates (T_i). Thus the payoff of strategy β during a harsh winter consisted only of offspring resulting from sexual reproduction until the date T_{end} :

$$W_s = \sum_{j=1}^{T_{\text{end}}} \left[\prod_{k=1}^{j-1} (1 - \beta_k) \cdot \lambda \right] \cdot \beta_j \cdot \lambda. \quad (1)$$

During a mild winter, when parthenogenetic females also were supposed to survive, their total number at the end of winter, W_p , was therefore

$$W_p = \left[\prod_{k=1}^{T_{\max}} (1 - \beta_k) \cdot \lambda \right], \quad (2)$$

and the total payoff of strategy β was $W_s + W_p$.

For each overwintering strategy β , fitness was calculated as the mean of equations (1) and (2) weighted by the probability that a mild (or harsh) winter occurs and by the probabilities of winter arrival ($P_i[T_{\text{end}}]$) defined for each date T_i of the time scale. Since this life-history trait refers to a long-term evolution in stochastic environment, geometric mean was used (Crow and Kimura 1970; Cooper and Kaplan 1982) because the weighted values vary

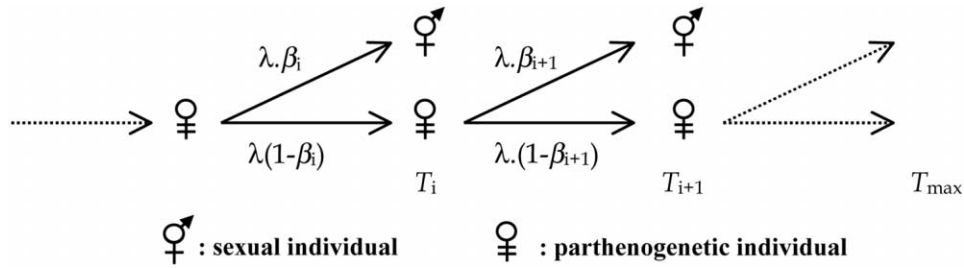


Figure 2: Schematic representation of sexual and parthenogenetic forms of reproduction in aphids. Overwintering strategies β are defined by the series of $\{\beta_i\}_{i \in \{1, \dots, 50\}}$ representing proportion of sexual individuals produced between two consecutive dates (T_i and T_{i+1}). See also table 1 for parameter definitions.

in time rather than in space (Moran 1992; Hopper 1999). Thus the global fitness was calculated as

$$G(\beta) = \prod_{i=1}^{T_{\max}} \left[(W_s + W_p)^{(1-p)} \cdot W_s^p \right]^{P_i(T_{\text{end}})} \quad (3)$$

The optimal strategy is defined as the strategy that maximizes equation (3) (Haccou and Iwasa 1995; Sasaki and Ellner 1995). It was calculated using numerical optimization (Satake et al. 2001), where the optimal solution was reached by means of a trial-and-error algorithm from any random starting strategy β . Three simulation runs, beginning from different starting points, were performed to check for the independence of the optimal strategy of initial conditions and paths.

There were three input variables: the rate of multiplication λ , winter severity p , and winter unpredictability

(defined by the standard deviation σ of the normal distribution describing the date of arrival of winter). For each combination of these parameters, two characteristics were calculated: the immediate production of sexual forms for a clone, $W_{S_i} = N_{i-1} \cdot (1 - \beta_i) \cdot \lambda$, which is the number of sexual individuals produced at T_i ($N_i =$ the number of individuals in the clone at T_i ; $N_i = \{\prod_{k=1}^{T_i} [1 - \beta_k] \cdot \lambda\}$), and the weighted immediate production of sexual forms for a clone W_{S_i}/W_s , which enables comparison of the shapes of curves describing the dependence of the proportion of sexuals on time for different combinations of input parameters. By keeping two parameters constant and varying the third, we studied the effect of each input variable on the optimal strategy. Only sequences of sexual production curves, W_{S_i}/W_s , which enable comparison with biological data and not the sequences of instantaneous investment in sexuals (vectors β), are presented, but results for β are the same.

Table 1: Model parameters

Term	Definition
λ	Number of offspring produced by one mother between two successive dates T_i and T_{i+1}
μ	Mean date of arrival of winter occurring at $T_{\max}/2$
σ^2	Variance of the date of arrival of winter
β_i	Proportion of sexual forms in the offspring of the parthenogenetic individuals at date T_i
$\beta = \{\beta_i\}_{i \in \{1, \dots, \max\}}$	Overwintering strategy of a clone
p	Probability that a cold winter occurs and therefore no parthenogenetic individual survives (called winter severity)
T_{end}	Date of arrival of winter
$P_i(T_{\text{end}})$	Probability that T_{end} occurs at date T_i
T_{\max} (set at T_{50})	Hatching time or arrival of spring (i.e., end of simulations)
W_s	Number of offspring resulting from sexual reproduction
W_p	Number of offspring resulting from parthenogenetic reproduction
$G(\beta)$	Global fitness of a clone adopting strategy β

Table 2: Number of years of aphid catches and number of years when daily and monthly temperatures were recorded in each site

Location	Number of years		
	Catches	Monthly temperatures	Daily temperatures
Aigre	11	23	23
Arras	19	22	22
Caen	10	13	13
Colmar	19	23	23
Landerneau	19	23	23
Montpellier	19	23	23
Orléans	19	22	22
Reims	10	17	17
Rennes	19	23	23
Valence	15	19	19
Versailles	12	16	16
Poznan	25	24	...
Dundee	22	28	...
Elgin	22	24	...
Kirton	16	16	...
Rothamsted	24	28	28
Starcross	24	20	14
Wye	24	29	...
Changins	14	14	...

Data

Data on weather conditions and aphid flights for several European locations are shown in table 2. We used monthly mean temperatures and, when available, daily maximum and minimum temperatures over a period of 14–29 yr (table 2). Meteorological data were obtained from the EXAMINE database for French, Swiss, and Polish sites and from the Biotechnology and Biological Sciences Research Council ARCMET database for the United Kingdom sites.

Aphid flight dynamics were obtained from suction traps (Macaulay et al. 1988) that caught flying aphids at about 12 m height by means of a constant 45 m³/min air flow. The catches belong to the AGRAPHID network (sites in France and Switzerland), to the Rothamsted Insect Survey and Scottish Agricultural Science Agency in the United Kingdom (fig. 3; Woiwod and Harrington 1994), and to the Instytut Ochrony Roslin in Poland, and they range from 11 to 24 yr of collection.

For each location the data consist of weekly counts of *R. padi* migrants in autumn (i.e., during the sexual reproduction period). Trapped aphids belonged to three different forms: winged parthenogenetic females, gynoparae, and males (fig. 1). Gynoparae and parthenogenetic females have a similar morphology and are thus hard to distinguish. The reproductive scheme of *R. padi* is panmictic, and the sex ratio was expected to be 1 : 1 (Fisher 1930).

Male catches have thus been considered as representative for the estimate of local production of sexual migrants. Assumption of the 1 : 1 sex ratio was therefore not crucial because assuming a different sex ratio would only mean multiplying all our estimates by the same constant. Male catches are also good estimators of the date of sexual reproduction because males migrate to the primary host to mate with oviparae.

The southern sites were excluded from calculations in which standard deviation was used because the small number of individuals (<200 males were caught per year in these sites compared with ~1,000 in the others) made the estimate unreliable.

Results

Relationships Predicted by the Model

Effect of the Rate of Multiplication. The rate of multiplication, λ , was varied from 1 to 3 because $\lambda \leq 1$ leads to population extinction and $\lambda > 3$ is biologically unrealistic because the population then reaches more than 10²⁵ in-

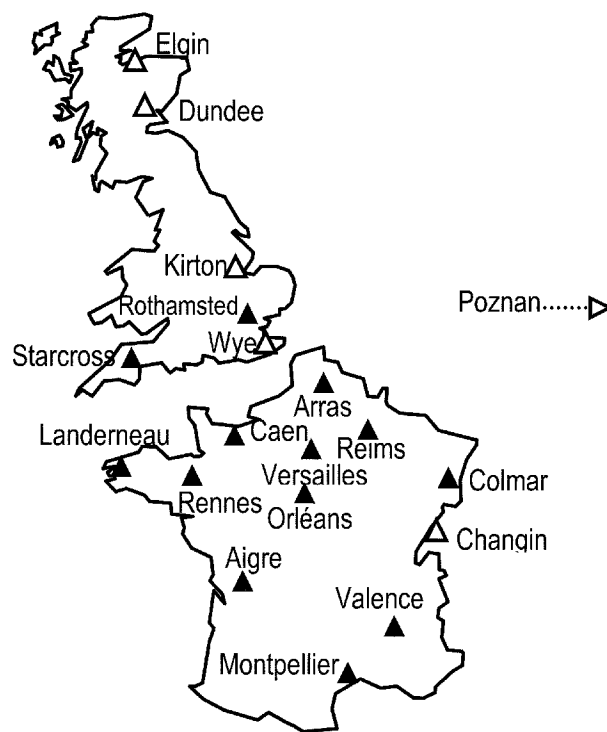


Figure 3: Locations of the suction traps in France and in the United Kingdom. Empty triangles are locations where only mean temperatures were measured. Filled triangles indicate locations where both mean and daily temperatures were measured. Number of years when biological and meteorological data were recorded is given in table 2.

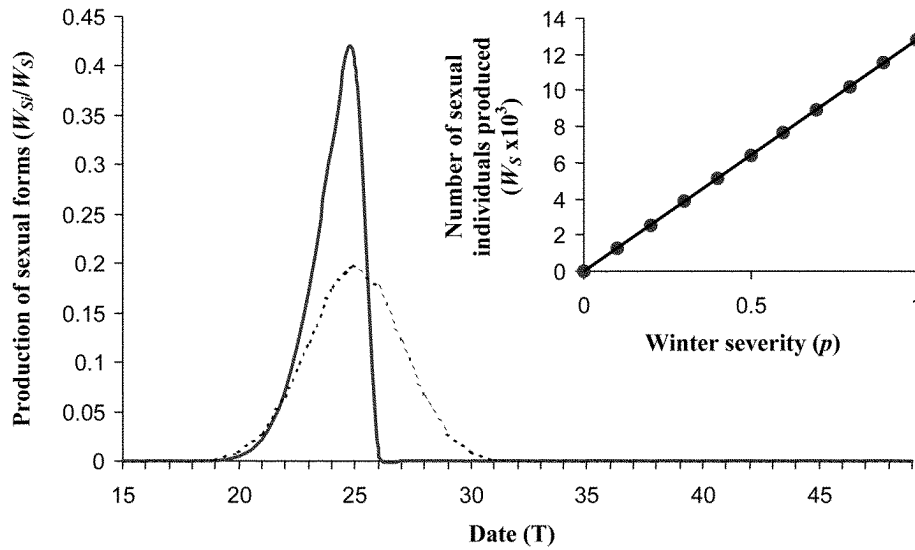


Figure 4: Model predictions of the temporal dependence of the weighted production of sexual forms (*solid line*) for different values of winter severity, p . The resulting curves are all identical and thus superimposed. Distribution of the date of arrival of winter is indicated by the dashed line. Parameters: $\mu = 25$ and $\sigma = 2$, $\lambda = 1.5$. *Inset*, Relationship between the total number of sexual individuals produced, W_s , and winter severity, p ($R^2 = 1$).

dividuals at T_{\max} . This parameter has no influence on the shape of the dynamic of production of sexual forms (data not shown); it only delays the time when sexual forms are produced (the higher the value of λ , the later the predicted optimum mean date when sexual morphs are produced).

However, the predicted optimum proportion of sexual individuals in the total number of offspring is negatively correlated with λ (data not shown). Thus, in addition to the well-known two-fold cost of sexuality due to production of both males and females necessary for the sexual reproduction (Maynard Smith 1971), our simulations indicate that sex becomes even less advantageous when parthenogenetic population growth rate is large.

Effect of Winter Severity. The probability that parthenogenetic individuals do not survive winter (winter severity, p) was ranged from 0 to 1. The optimal sequence of production of sexual forms predicted by the model was independent of p (fig. 4), since p acts only on the amplitude of the rough production of sexuals (data not shown). Conspicuous is the linear increase in the total number of sexual individuals produced (W_s) as a function of p (fig. 4, *inset*). In the extreme cases, no sexual individuals are produced when parthenogenetic females always survive winter ($p = 0$), and only sexuals are produced when winter is harsh every year ($p = 1$).

The global investment in the sexual reproduction of a

clone I , calculated as the sum of all immediate investments is

$$I = \sum_{j=1}^{T_{\max}} \left[\prod_{k=1}^{j-1} (1 - \beta_k) \right] \cdot \beta_j. \quad (4)$$

Our simulations yielded $I = p$.

Effect of Winter Unpredictability. Figure 5 shows model predictions for standard deviation in the date of arrival of winter, σ (winter unpredictability), when $0 \leq \sigma \leq 6$. For $\sigma > 6$, the normal distribution gives a nonnegligible probability of arrival of winter before 0 and after T_{\max} , which might yield border effects. The deterministic case, $\sigma = 0$ (not depicted in fig. 5), leads of course to a unique event of production of sexual individuals at date T_{25} . When stochasticity is added ($\sigma > 0$), production of sexual forms becomes spaced out over several dates (fig. 5). Increase of stochasticity (σ) results in flattening of the curves. For $0 < \sigma < 2$, the peak of production of sexual individuals occurs before the mean date of the arrival of winter, while for $\sigma \geq 2$ the peak of production of sexual individuals occurs after this date. Skewness of the curves is negatively correlated, while position of the peak is positively correlated with σ (i.e., peaks occur later and skewness becomes increasingly negative when σ increases). The dependence of the standard deviation of the curves on winter unpre-

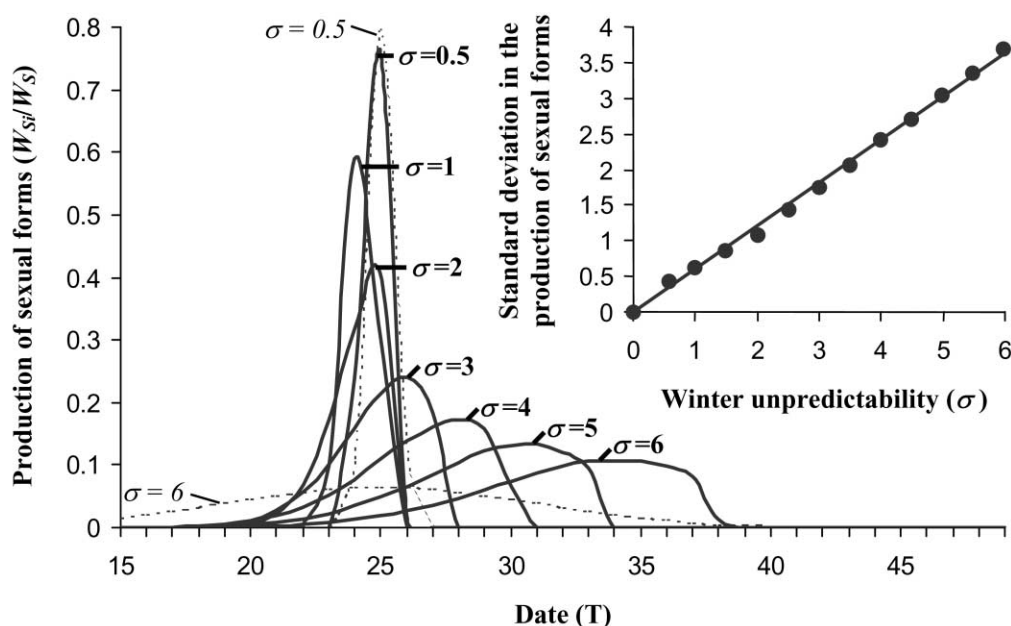


Figure 5: Model results showing the temporal dependence of the weighted production of sexual forms (*solid lines*) when winter unpredictability (σ [standard deviation of the date of arrival of winter], indicated by the numbers at the curves) varies. Distributions of the date of arrival of winter are indicated by dashed lines for extreme values of σ ($\sigma = 0.5$ and $\sigma = 6$). Parameters: $\mu = 25$, $\lambda = 1.5$, $p = 0.2$. *Inset*, Relationship between standard deviation in the production of sexual morphs and standard deviation of the date of arrival of winter ($R^2 = 1$).

dictability is presented in the inset. This linear relationship shows that variability in the response (i.e., in the date of production of sexuals) can be adaptive (because the inset results from an optimization procedure) and may be considered as a support for the existence of adaptive stochastic polyphenism.

Tests of the Theoretical Predictions against Observed Relationships

Number of Males against Winter Severity. For sites where daily meteorological data were available (table 2), we calculated the index of winter severity as a between-years mean number of days for which minimal temperature was below -5°C , a temperature that mature aphids are unlikely to survive for long (Griffiths and Wratten 1979; Dedryver and Gellé 1981; Butts et al. 1997). The mean numbers of trapped *Rhopalosiphum padi* males were highly significantly positively correlated with the index of winter severity (fig. 6; $R^2 = 0.60$, $P = .001$), which supports the theoretical prediction of the positive correlation between the number of sexual individuals produced and winter severity (fig. 4, *inset*). This prediction is further supported by the well-known fact that anholocyclic clones are more abundant in the oceanic climate and in the south, whereas

holocyclic clones prevail in the continental and northern sites where the winter climate is more severe (Dedryver 1983; Pons et al. 1992, 1995; Harrington et al. 1995; Zhou et al. 1995; Simon et al. 1996; Rispe et al. 1998a).

Date of Peak Male Production against Winter Timing. To estimate T_{end} , the date of arrival of winter, we performed linear regressions of monthly mean temperatures from August to December against time for each year and site and determined T_{end} for each site as the mean time when the regression lines crossed 12°C because this temperature is known to induce sexual reproduction in *R. padi* (Dixon and Glen 1971; Simon et al. 1991). We found a highly significant positive correlation between the mean date of male catches in different suction traps and date of arrival of winter (fig. 7; $R^2 = 0.69$, $P < .001$). This supports the model predictions because it is obvious that, if everything else is equal, there is a linear relationship between the date of the peak of male flight in our model and the value of μ (data not shown). Figure 7 also shows conspicuous north-south (Montpellier vs. Elgin) and oceanic versus continental climate (Rennes vs. Poznan) gradients, consistent with the south-north gradient observed by Clark et al. (1992) and the NE-SW gradient observed by Rispe et al. (1998a).

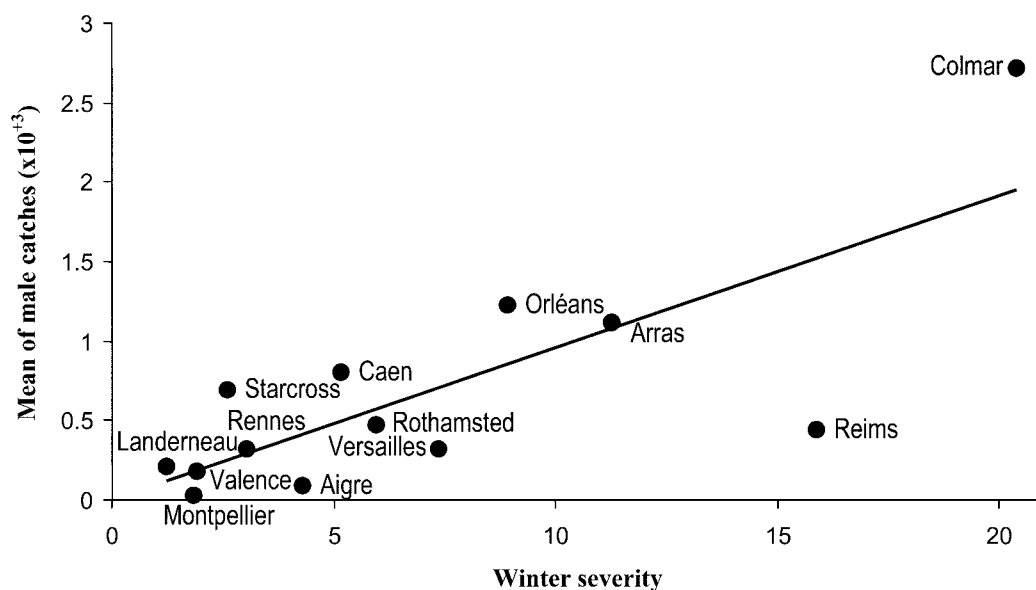


Figure 6: Relationship between mean total number of males of *Rhopalosiphum padi* caught each year in suction traps and winter severity (the mean number of days where the minimum temperature was below -5°C) in various sites ($R^2 = 0.60$, $P = .001$).

Variance in the Male Flight Curves against Winter Unpredictability. We correlated the mean standard deviation of the dates of male catches at each site with winter unpredictability, calculated as the standard deviation of the date of arrival of winter in the considered site as calculated in the previous section (fig. 8; $R^2 = 0.44$, $P = .006$). High altitude and continental sites (Changins, Poznan, and Colmar) represent sites with good winter predictability linked with a typically narrow within-year flight peak (small mean standard deviation of the dates of male catches) as opposed to locations close to the ocean (Landerneau, Rennes, Dundee, or Elgin), which show large winter unpredictability. These results are fully consistent with the model prediction concerning stochastic polyphenism (fig. 5, *inset*).

Moreover, we found only a weak and not significant correlation between winter unpredictability and the mean date of arrival of winter in our meteorological data ($R^2 = 0.17$, $P = .12$). Thus aphid flights seem to be adapted to the variability in the date of arrival of winter, and the relationship in figure 8 is not just a simple consequence of adaptation to the mean date of arrival of winter depicted in figure 7.

Discussion

We have shown that stochastic environmental fluctuations lead to spreading the production of sexual forms over a time interval if the date of the arrival of winter is unpre-

dictable, which supports an adaptive mechanism for the plasticity of this trait. When the population growth rate is large and weather is unpredictable, then it is advantageous to invest in parthenogenesis for as long as possible but simultaneously produce some sexual individuals far before the peak. The dependence of the proportion of sexuals on time is therefore described by a curve with a large variance, negative skewness, and the peak for the production of sexual morphs close to the ultimate date when reproduction stops completely. Similar results were obtained by Satake et al. (2001) for plants. Contrary to our model, they limited the population growth rate in considering that the reproductive success is a function of the timing of reproduction. This indicates that patterns described above are general and probably independent of the type of density dependence.

The global investment in sexual reproduction of a clone, I , defined in equation (4), is equal to p (the probability of occurrence of a harsh winter year) in our simulations. This is consistent with Rispe et al.'s (1998b) prediction that the ratio of gambler strategy over saver strategy in a population at the evolutionarily stable strategy, α^* , should depend on the severity of winter, since $\alpha^* = p$. However, in their article, within-population variability results from genetic polymorphism of two pure strategies (i.e., a mixing of gambler and saver genotypes), whereas our model allows a single genotype to invest stochastically both in the parthenogenetic and in sexual reproduction simulta-

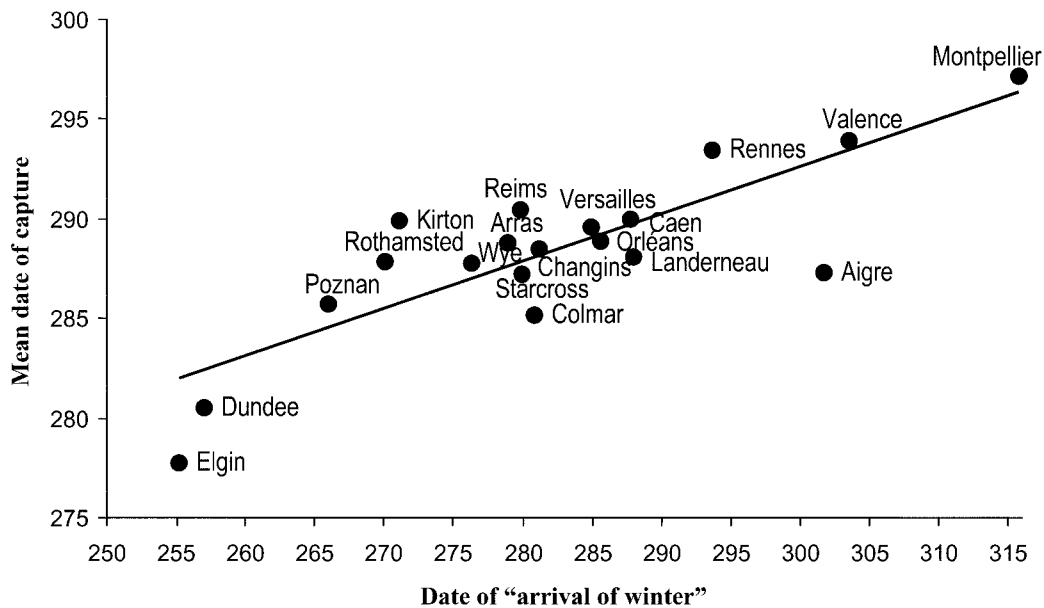


Figure 7: Relationship between the mean date of capture of males of *Rhopalosiphum padi* each year in suction traps and the date of arrival of winter in various sites ($R^2 = 0.69$, $P < .001$).

neously. The Cohen model (1966) applied to plant dormancy also predicts prolonged dormancy proportion, which matches the probability of bad years. However, such equality can disappear when complicating the model (Clausen and Venable 2000, from biological data; Menu et al. 2000).

The data did not provide absolute evidence for stochastic polyphenism. Alternatively, the coexistence of pure strategies consisting of a deterministic time of switching from the parthenogenetic to the sexual mode of reproduction could account for the observed variability. However, first, our model allowed the selection of a pure strategy. This was never observed except in the case of a constant date of arrival of winter (absence of uncertainty). This proved that stochastic polyphenism is generally a better strategy than any pure deterministic one.

Second, in our opinion, three main factors could result in the maintenance of a mixture of pure strategies: a local selection followed by a large-range dispersal, a frequency-dependent selection, or a fluctuating selection. Aphids have the ability to disperse on a large scale. However, the strong correlation between the mean date of capture and the mean date of arrival of winter (fig. 7) suggests the predominance of local adaptation over mixing by dispersal. Similarly, it is not clear why dispersal could lead to the observed adaptation to winter unpredictability (fig. 8). Additionally, it is also not clear why frequency dependence would result in these observed correlations. Thus, a fluctuating selection appears to be the most serious alternative

explanation. However, it would probably require the date of switching being under the control of a relatively complex genetic system (e.g., series of genetic factors with a hierarchy of dominance, with the factors that determine an early date of switching being dominant to those that determine a later date, allowing the latter to be sheltered in heterozygote individuals in years when winter arrives earlier). In addition, such a fluctuating selection mode would result in a variation in relative frequencies of the strategies from year to year (Rispe et al. 1998a). In particular, at a given site, the observed mean date of capture of males at year n would be expected to be sooner when the arrival of winter had been early the year before ($n-1$). A variance-covariance analysis has shown that the observed mean date of capture of males in year n was highly dependent on the site ($P < .0001$) but not on the date of arrival of winter in the same site the previous year ($P = .3952$).

Finally, a higher variability in the date of production of sexuals could result from a global higher genetic variability in the population that could result in particular from the relative importance of the sexual reproductive mode. However, this variability appeared lower in continental sites (Colmar, Changins, Poznan), where aphids are considered to be mostly holocyclic, than in sites with an oceanic climate (Rennes, English sites), where anholocyclic clones are considered to be predominant.

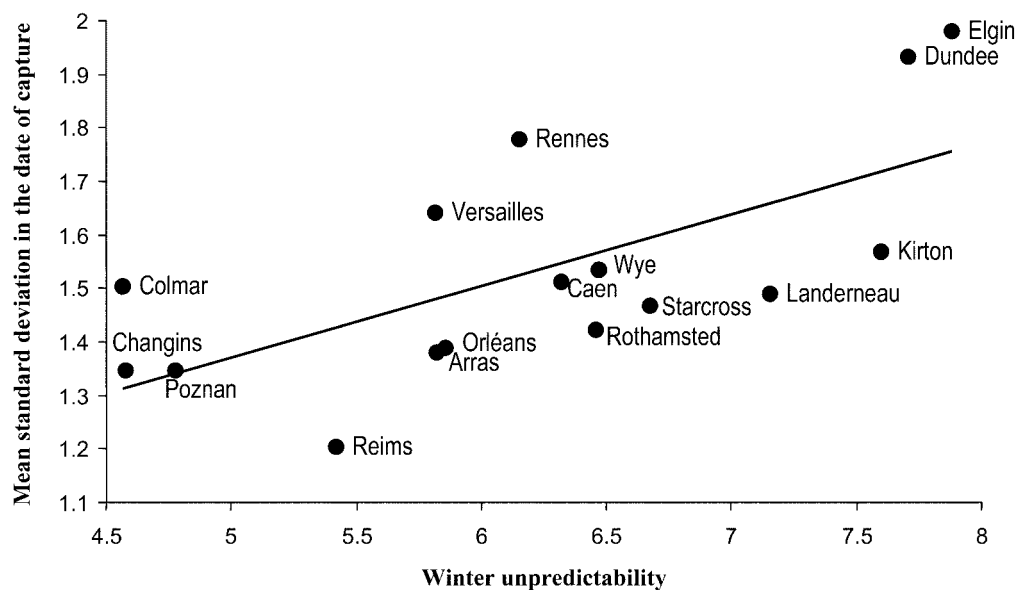


Figure 8: Relationship between the mean standard deviation in the date of capture of males of *Rhopalosiphum padi* caught each year in suction traps and winter unpredictability (standard deviation in the date of arrival of winter) in various sites ($R^2 = 0.44$, $P = .006$).

All of those considerations suggest that stochastic polyphenism is more parsimonious and more likely than a coexistence of pure strategies to explain the observed relationship between environmental variability and variability in the date of male capture of *Rhopalosiphum padi*. Of course, we do not pretend that local populations are made of a single genotype. However, observed data are consistent with our model and could thus result from the tendency to select for a higher phenotypic variability where the environment is more variable.

Experimental data on various aphid species show that the main cues for the decision to end the sequence of parthenogenetic generations are temperature and day length (MacGillivray and Anderson 1964; Dixon and Glen 1971; Tsitsipis and Mittler 1976, 1977a, 1977b), but their relative importance is less clear. In their model, Ward et al. (1984) predict the precise time of the switch to production of sexual forms on the basis of the temperatures experienced. Their predictions conform to our empirical data in terms of the correlation between the means (mean peak of male production being correlated with the mean date of arrival of winter) but are lacking the stochastic aspect. Inclusion of stochasticity is thus our main new contribution to the specific problem of aphid reproduction timing.

We found that the peak of flight activity is negatively correlated with average winter severity, consistent with Clark et al. (1992), who found a latitudinal trend in the dates of male flights in two aphid species. Thus, timing

of production of sexual forms depends on average climatic conditions of the site rather than on the severity of the winter experienced the year before, as investigated by Rispe et al. (1998a), although exceptions are possible (Harrington et al. 1995).

To summarize, the most important message from this article is that the existence of stochastic polyphenism as an adaptive strategy can be tested on real data by means of correlation between phenotypic variability and predictability of the environment. Some of the predictions of our model are not new (correlation between the mean date of male flight and latitude or characteristic temperature, correlation between winter severity and investment in sexuals), but they are tested against our extensive set of data, which enabled us to achieve much more significant results than before. As shown above, environmental factors are known to be used as cues for the decisions. However, environment predictability can only be partial, and the best strategy may be a mixing of both predictive and coin-flipping plasticity, as shown by Danforth (1999) and Clauss and Venable (2000) for dormancy strategies. The variance-to-variance correlation (i.e., variance in the date when sexual individuals are produced against variance in the date of arrival of winter) has never been demonstrated before. Neither has it been shown that absence or presence of this correlation enables distinction between nonadaptive random variation in observations and adaptive coin-flipping plasticity.

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