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# Clonal variability in sequences of morph production during the transition from parthenogenetic to sexual reproduction in the aphid *Rhopalosiphum padi* (Sternorrhyncha: Aphididae)

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**Key words.** Aphididae, *Rhopalosiphum padi*, geographic clones, overwintering, reproductive strategy, parthenogenetic, sexual, diapausing eggs, photoperiod, thermoperiod

**Abstract.** Winter climate determines the success of the two main reproductive strategies employed by aphids. Permanent parthenogens survive as parthenogenetic females in mild winters, but are regularly eliminated by low temperatures; while cyclical parthenogens, which switch to sexual reproduction by the end of summer, produce every year fertilised diapausing eggs resistant to frost.

We have studied the variation in sexual morph production of several clones of the cereal aphid *Rhopalosiphum padi* (L.) showing both strategies. Twenty clones of this species differing by their geographic origin and their mode of reproduction were placed in two laboratory environments mimicking the changes of photoperiod and thermoperiod occurring naturally from the end of summer and during the autumn in oceanic and continental conditions. The analysis of clonal responses in both climatic conditions showed (i) a wide variation in investment of clones in sexual reproduction with, in particular, evidence for a mixed strategy employed by clones producing both sexuals without ceasing parthenogenetic reproduction, (ii) no geographic adaptation among clones belonging to cyclical parthenogenetic populations, (iii) an earlier production of sexuals in continental conditions and a higher production of males in oceanic conditions.

Furthermore, we have compared the dates of first appearance of sexuals in our experiments with those occurring in the field based on a suction trap database and found that sexuals were caught in nature at least four weeks earlier than in the lab. These results underline the need for a better understanding of the influence of the whole array of environmental factors inducing the transition from parthenogenetic to sexual reproduction in aphids.

## INTRODUCTION

Overwintering is one of the most difficult tasks any insect has to face during its life cycle (Leather et al., 1993). Many strategies are employed by insects to survive this season. In aphids, two main strategies occur among populations, whose success is determined by winter climate: permanent parthenogens survive as parthenogenetic females in mild winters but are regularly eliminated by low temperatures while cyclical parthenogens switch to sexual reproduction by the end of summer and produce fertilised diapausing eggs resistant to frost (Dixon, 1987; Leather, 1992).

In cyclical parthenogenetic populations of many host alternating aphid species, the parthenogenetic phase occurs during the spring and summer, on herbaceous plants (secondary hosts), and sexual reproduction occurs on a woody plant (the primary host). At the end of summer and the beginning of autumn, two morphs are responsible for the migration to the primary host: gynoparae and males. Gynoparae are winged parthenogenetic females, giving birth on the primary host to oviparous sexual females. Winged parthenogenetic females or virginoparae, are also produced during the autumn by permanent parthenogenetic populations. In *Rhopalosiphum padi* (L.), a species alternating between Poaceae (secondary hosts) and *Prunus padus* L. (primary host) both overwintering strategies, cyclical and permanent parthenogenesis, occur among populations, especially in oceanic regions (Dedryver & Gellé, 1982; Simon et al., 1991). One strategy or the other may be locally favoured depending on the environmental conditions. In France, sexual reproduction is more widespread in the eastern regions where winters are regularly cold and *P. padus* abundant, while parthenogenetic overwintering is favoured in the western regions (Simon et al., 1996a). Furthermore, a third strategy, termed androcycly, is employed by some clones which are permanent parthenogens but still produce males as well as parthenogenetic females (Simon et al., 1991).

The timing of the switch to the sexual phase is important for a clone to maximise fitness. The later cyclical parthenogenetic clones switch to sexual reproduction, the greater their fitness, because the growth season is longer. Nevertheless, these clones are constrained to switch to the sexual phase before the leaf fall of the primary host on which eggs are laid. All individuals involved in sexual reproduction have to be produced synchronously between clones to ensure the mating rendez-vous.

For host alternating species, the decision to end the parthenogenetic reproduction and to switch to the sexual phase must be taken at least by the mother of the gynoparae and the males (Ward et al., 1984). Together, short day-lengths and low temperatures induce the development of both gynoparae and males in species like *R. padi* (Dixon & Glen, 1971), *Myzus persicae* (Sulzer) (Black-

man, 1975), *Dysaphis plantaginea* (Passerini) (Bonnemaison, 1970). Aphids may however show a variability in their responses to these environmental cues. For instance, two clones of *Acyrthosiphon pisum* (Harris) collected in the same field showed a one hour difference in the photophase at which mating females first appear (MacKay, 1989). Such variability may represent an adaptative significance because the length of the growing season is not predictable in temperate climates.

Regional variation in the timing of the first males of *R. padi* caught in suction traps were reported in Great Britain and related to photoperiodic differences among sites. Males were recorded earlier in the North (Tatchell, 1988), and these observations were confirmed experimentally. Clones from the North of Great Britain switch to sexual reproduction earlier than clones from southern regions (Austin et al., 1996). Results from these authors suggested also that the effect of photoperiod was modulated by temperature.

Concerning *R. padi*, several questions remain open. When should a clone switch to sexual reproduction in the field? Is there any clonal variability in the responses to conditions inducing the production of sexuals? Is there any geographic adaptation of the responses to these inducing conditions? In order to answer these questions, several clones of *R. padi* differing by their geographic origin and their overwintering strategy were placed in two laboratory environments mimicking the change of photoperiod and thermoperiod occurring naturally from the end of summer in oceanic and continental conditions. Finally, experimental results concerning the timing of sexual morph production of these clones were compared with field observations.

## MATERIAL AND METHODS

### Aphid clones

Four groups of five clones of *R. padi* combining different geographic origins and overwintering strategies (mode of reproduction) were tested (Table 1). These clones were collected before the spring migration in three regions of France differing in winter climate. Clones of group 1 were collected on primary hosts in a continental region (Colmar, eastern France). They were previously characterized as cyclical parthenogens, analysing their response to sexual morph inducing conditions (15°C and 10L : 14D) (Simon et al., 1991). They were also characterized for their mitochondrial DNA and carried haplotype II generally associated with cyclical parthenogenetic clones (Martinez-Torres et al., 1996; Simon et al., 1996b). Their sex-ratio, defined as the ratio *gynoparae / (males + gynoparae)* was also determined in a previous experiment using the same reference standard conditions (Rispe et al., 1999). Values ranged between 0.30 and 0.92.

Clones of group 2 were collected on primary hosts in an oceanic region (Rennes, western France). These clones were also characterized as cyclical parthenogens and carried haplotype II for mtDNA. Their sex-ratio ranged between 0.27 and 0.93.

Clones of group 3 were collected on secondary hosts in northern France. The climate of this region is considered as intermediate between those of eastern and western France with a mixture of cold and mild winters. The life cycle of these clones was unknown before the experiment but they carried haplotype II for mtDNA, typical for cyclical parthenogenetic clones.

TABLE 1. Origin of *Rhopalosiphum padi* clones investigated for sexual morph production. (Data on overwintering strategy and mitochondrial DNA haplotype from Simon et al., 1996a and Martinez-Torres et al., 1997).

| Clones | | Collection | | | Overwintering strategy | mtDNA haplotype |
|---|---|---|---|---|---|---|
| Group | Name | Date | Site | Host-plant | | |
| 1 | Rp1 Colmar | 04/23/92 | Colmar (eastern France) | Bird cherry (primary host) | Sexual reproduction | H II |
| | Rp3 Colmar | 05/05/92 | | | | |
| | Rp4 Colmar | 04/23/92 | | | | |
| | Rp10 Colmar | 04/28/92 | | | | |
| | Rp15 Colmar | 04/27/92 | | | | |
| 2 | Rp1 Arbo | 04/13/92 | Rennes (western France) | Bird cherry (primary host) | Sexual reproduction | H II |
| | Rp8 Arbo | 04/06/92 | | | | |
| | Rp18 Arbo | 04/21/92 | | | | |
| | Rp1 Rebillon | 04/06/92 | | | | |
| | Rp7 Ensa | 04/13/92 | | | | |
| 3 | Rp2 Tilloy | 04/12/94 | Tilloy (northern France) | Poaceae (secondary host) | Unknown | H II |
| | Rp3 Tilloy | 04/12/94 | | | | |
| | Rp4 Tilloy | 04/12/94 | | | | |
| | Rp6 Tilloy | 04/12/94 | | | | |
| | Rp9 Tilloy | 04/12/94 | | | | |
| 4 | Rp K 93 | 02/28/90 | Rennes (western France) | Poaceae (secondary host) | Androcycle | H I |
| | Rp 15 Rennes | 02/16/90 | | | | |
| | Rp 22 Rennes | 02/16/90 | | | | |
| | Rp 26 Rennes | 02/26/90 | | | | |
| | Rp 32 Rennes | 02/16/90 | | | | |

Clones of group 4 were collected on secondary hosts in an oceanic region (Rennes, western France). These clones were characterized as androcyclic and carried haplotype I for mtDNA which is exclusively found among permanent parthenogenetic clones.

After collection and before experiment, the 20 clones were maintained at 20°C and a light regime of 16L : 8D on wheat seedlings (cv. Arminda) to ensure continuous parthenogenetic reproduction (Simon et al., 1991).

**Experiments**

Photoperiodic and thermoperiodic conditions

In order to mimick changes in photoperiod and thermoperiod at the end of summer and during autumn, light and temperature day regime recorded for a mild season at Rennes (oceanic condition) and a cold season at Colmar (continental condition) were simulated in two programmable cabinets from August 15th (before the beginning of sexuals production) to November 30th (after the end of sexuals production). The simulated photoperiodic decrease corresponded to the latitude of Rennes and Colmar (48°07′N). Diurnal and nocturnal temperatures were applied during photophase and scotophase respectively. To simulate the mild oceanic condition, the diurnal temperature was equal to the mean day maxima calculated from 1978 to 1995 at Rennes plus 2°C and the nocturnal temperature was equal to the mean day minima calculated from 1978 to 1995 at Rennes plus 1.5°C. To simulate the cold continental condition, the diurnal temperature was equal to the mean day maxima calculated from 1978 to 1995 at Colmar minus 2°C and the nocturnal temperature was equal to the mean day minima calculated from 1978 to 1995 at Colmar minus 1.5°C (Fig. 1). For technical reasons, the lowest temperature programmed in the cabinets was limited to 2°C.

Aphid rearing

Both experiments simulating oceanic and continental conditions were started with ten fourth instar alatiform larvae of each of the 20 clones. Aphids were placed in Perspex boxes (one clone per 14 × 9 × 40 cm box) and reared on wheat seedlings (cv. Arminda) grown in square plastic pots 7 × 7cm (approximately 40 seeds per pot). Plants were changed every two weeks to provide good quality food and to limit fungal development. Old and new pots were left together within the Perspex box during three days to allow infestation of the new pots.

Twice a week, all mature winged aphids were removed from Perspex boxes with a fine brush and identified as virginoparae, males or gynoparae. Gynoparae and virginoparae were distinguished with the squash blot test (Lowles, 1995) periodically controlled with choice tests adapted from Tachell et al. (1988).

**Field observations**

Field observations came from the suction traps network Agraphid which has been operating in France since 1978 (Hullé, 1991). Because of practical reasons, gynoparae were not distinguished from winged virginoparae in catches. The comparison between experimental results and field observations was therefore limited to male catches. Data collected from 1978 to 1995 at Colmar, Rennes and Arras-Loos, corresponding respectively to the eastern, western and northern regions were used in this study.

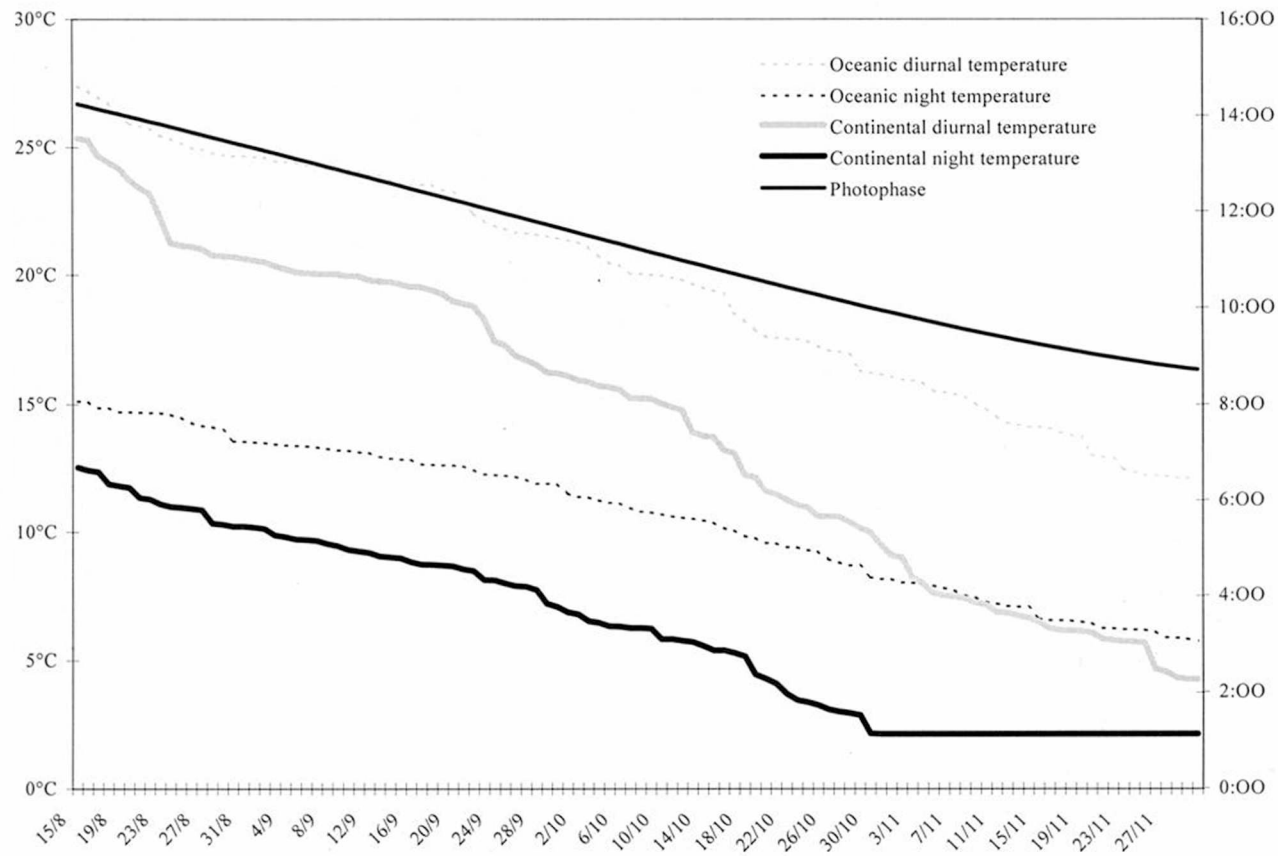


Fig. 1. Simulated temperature and photoperiod conditions. Diurnal temperature was applied during photophase and night temperature during scotophase.

### Statistical analysis

Sexual production and variability in clonal responses

Clonal responses were described with seven variables: the date of the first gynoparae (dg1) and the first male (dm1), the date when the first five percent of gynoparae (dg5) and males (dm5) were produced, the total number of gynoparae (gtot), males (mtot) and winged virginoparae (vtot). All temporal variables (dg1, dg5, dm1, dm5) corresponded to simulated dates. Effects of group (geographic origin combined with overwintering strategy) and of experimental condition were tested using a two-way analysis of variance performed on the whole set of variables (SAS, 1988). In order to test the homogeneity of responses within a group the effect of clone nested within groups was added. Comparisons of means for the two main effects, group and condition, were also performed using the Duncan's multiple range test (SAS, 1988). All sequences were then aggregated in a few types following a classification based on principal component analysis of the above variables (STATlab, 1995).

The hypothesis of geographic adaptation among cyclical parthenogenetic clones collected on primary hosts (group 1 and 2) was tested by an analysis of variance where the effects of the geographic origin (East/West), the experimental condition and the interaction of both factors were included in the model (SAS, 1988).

Experimental conditions of sexual morph production and comparison with field data

Temperatures and photoperiod corresponding to adult appearance (dg1, dm1, dg5 and dm5) were recorded. The corresponding dates of larviposition were also estimated to infer the birth of sexuals. For that, a day degree model was applied to calculate a development rate $\frac{1}{t_D} = \frac{1}{K}(T - T_0)$ where $t_D$ was the developmental time, $K$ a constant, $T$ the ambient temperature (day mean temperature weighted by L : D ratio) and $T_0$ was the lowest temperature for development. Developmental times of winged adults of *R. padi* gave K= 136 day · °C and $T_0$ = 6.1°C (Dean, 1974; J.-C. Simon, unpubl.). The experimental values of dg1 and dm1 were directly compared to the date of first appearance of males in suction trap catches. The corresponding dates of larviposition were also compared in order to correct the differences of temperature between controlled and field conditions.

## RESULTS

### The different sequences of sexual morph production

Variability in clonal responses within group

Variability in clonal responses within groups was low. Only two variables (dg1 and vtot) out of seven showed significant differences (Table 2). This variability observed for dg1 was mainly due to clones Rp 1 Arbo or Rp 10 Colmar which showed a larger variability than other clones (Table 3). For vtot the source of inter-clonal variability was principally due to clone Rp 6 Tilloy which produced a higher number of virginoparae in the oceanic condition than in the continental condition.

Variability in clonal responses between group

The group effect was significant for all variables (Table 2). Gynoparae were produced in each of the four groups but in different ways. First gynoparae (dg1) were detected early in group 1 and 2 (September 21st and 25th respectively), then in group 3 (October 1st) and much later in group 4 (November 28th). The first 5% of gynoparae (dg5) were reached at the same date (October 3rd) in groups 1 and 2, nine days later in group 3 and at the end of November in group 4. Total production of gynoparae (gtot) was lowest in group 4 with 12 individuals on average instead of hundreds in other groups. The main result concerning male production was the difference observed between group 4 and the others. In this group, males were produced significantly later (dm1 = November 11th and dm5 = November 20th) than in other groups for which dm1 ranged from October 21st to October 26th and dm5 from October 30th to November 2nd. The total number of males produced (mtot) was also lowest in group 4. Conversely, group 4 produced the highest number of winged virginoparae (vtot = 2769), while group 1 and 2 showed the lowest production (vtot = 278 and 302 respectively). The production of winged virginoparae was also important in group 3 with an average value of 2043 individuals.

TABLE 2. Analysis of variance and Duncan's test of comparison of means. dg1, dm1: dates of the first gynoparae and males; dg5, dm5: dates of the first 5% of gynoparae and males; gtot, mtot and vtot: total numbers of gynoparae, males and virginoparae; *(Pr > F) < 0.05, **(Pr > F) < 0.01, ***( Pr > F) < 0.001; means with the same letter are not significantly different.

| | dg1 | | dg5 | | gtot | | dm1 | | dm5 | | mtot | | vtot | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| F value | | | | | | | | | | | | | | |
| group | 522.43 | *** | 130.80 | *** | 40.05 | *** | 8.58 | ** | 4.27 | * | 5.36 | ** | 200.16 | *** |
| condition | 57.53 | *** | 63.27 | *** | 1.63 | | 1.94 | | 0.02 | | 16.44 | *** | 35.36 | *** |
| clone within group | 4.81 | ** | 2.32 | | 1.93 | | 2.02 | | 0.61 | | 1.20 | | 2.95 | * |
| Comparison of means | | | | | | | | | | | | | | |
| group 1 | 21 Sept | d | 03 Oct | c | 740 | b | 26 Oct | b | 31 Oct | b | 146 | a b | 278 | c |
| group 2 | 25 Sept | c | 03 Oct | c | 1112 | a | 24 Oct | b | 02 Nov | b | 326 | a | 302 | c |
| group 3 | 01 Oct | b | 12 Oct | b | 494 | c | 21 Oct | b | 30 Oct | b | 250 | a | 2043 | b |
| group 4 | 26 Nov | a | 26 Nov | a | 12 | d | 11 Nov | a | 20 Nov | a | 6 | b | 2769 | a |
| Continental condition | 29 Sept | b | 05 Oct | b | 543 | a | 25 Oct | a | 01 Nov | a | 61 | b | 1084 | b |
| Oceanic condition | 06 Oct | a | 19 Oct | a | 636 | a | 26 Oct | a | 04 Nov | a | 303 | a | 1613 | a |

TABLE 3. Sexual morph production of *Rhopalosiphum padi* clones; the variables dg1, dg5, gtot, dm1, dm5, mtot and vtot are defined in Table 2. Letters in column "type" correspond to six classes following a principal component analysis performed on these variables.

| Condition | Group | Clone | dg1 | dg5 | gtot | dm1 | dm5 | mtot | vtot | type |
|---|---|---|---|---|---|---|---|---|---|---|
| Continental | | Rp 1 Colmar | 15 Sept | 19 Sept | 426 | 16 Oct | 3 Nov | 29 | 114 | *a* |
| | | Rp 3 Colmar | 15 Sept | 19 Sept | 776 | 19 Oct | 24 Oct | 36 | 23 | *a* |
| | 1 | Rp 4 Colmar | 26 Sept | 29 Sept | 839 | 21 Nov | 21 Nov | 1 | 260 | *c* |
| | | Rp 10 Colmar | 8 Sept | 19 Sept | 513 | 23 Oct | 24 Oct | 30 | 99 | *a* |
| | | Rp 15 Colmar | 19 Sept | 29 Sept | 1048 | – | – | 0 | 226 | *d* |
| | | Rp 1 Arbo | 19 Sept | 26 Sept | 1121 | 16 Nov | 17 Nov | 8 | 15 | *c* |
| | | Rp 8 Arbo | 15 Sept | 22 Sept | 1133 | 23 Oct | 7 Nov | 317 | 358 | *a* |
| | 2 | Rp 18 Arbo | 19 Sept | 26 Sept | 1045 | – | – | 0 | 186 | *d* |
| | | Rp 1 Rebillon | 12 Sept | 15 Sept | 1044 | 12 Oct | 31 Oct | 259 | 92 | *a* |
| | | Rp 7 Ensa | 2 Oct | 1 Oct | 848 | 30 Oct | 10 Nov | 149 | 0 | *c* |
| | | Rp 2 Tilloy | 29 Sept | 3 Oct | 342 | 19 Oct | 20 Oct | 17 | 1774 | *e* |
| | | Rp 3 Tilloy | 26 Sept | 13 Oct | 670 | 19 Oct | 3 Nov | 195 | 2363 | *e* |
| | 3 | Rp 4 Tilloy | 29 Sept | 6 Oct | 293 | 26 Oct | 27 Oct | 5 | 1297 | *e* |
| | | Rp 6 Tilloy | 29 Sept | 3 Oct | 336 | 16 Oct | 20 Oct | 149 | 1359 | *e* |
| | | Rp 9 Tilloy | 29 Sept | 6 Oct | 393 | 16 Oct | 17 Oct | 15 | 1151 | *e* |
| | | Rp K 93 Rennes | 22 Nov | 22 Nov | 25 | 7 Nov | 7 Nov | 6 | 2549 | *f* |
| | | Rp 15 Rennes | 28 Nov | 28 Nov | 13 | – | – | 0 | 2084 | *f* |
| | 4 | Rp 22 Rennes | – | – | 0 | – | – | 0 | 2411 | *f* |
| | | Rp 26 Rennes | – | – | 0 | – | – | 0 | 2208 | *f* |
| | | Rp 32 Rennes | – | – | 0 | – | – | 0 | 3124 | *f* |
| Oceanic | | Rp 1 Colmar | 25 Sept | 10 Oct | 815 | 18 Oct | 27 Oct | 269 | 450 | *b* |
| | | Rp 3 Colmar | 22 Sept | 10 Oct | 666 | 18 Oct | 27 Oct | 625 | 446 | *b* |
| | 1 | Rp 4 Colmar | 2 Oct | 17 Oct | 624 | 25 Oct | 27 Oct | 13 | 338 | *c* |
| | | Rp 10 Colmar | 22 Sept | 13 Oct | 544 | 18 Oct | 24 Oct | 452 | 333 | *b* |
| | | Rp 15 Colmar | 28 Sept | 17 Oct | 1158 | 12 Nov | 12 Nov | 2 | 496 | *c* |
| | | Rp 1 Arbo | 4 Oct | 17 Oct | 1418 | 18 Oct | 3 Nov | 675 | 287 | *b* |
| | | Rp 8 Arbo | 28 Sept | 3 Oct | 868 | 22 Oct | 31 Oct | 365 | 782 | *c* |
| | 2 | Rp 18 Arbo | 28 Sept | 6 Oct | 2263 | 25 Oct | 27 Oct | 17 | 691 | *b* |
| | | Rp 1 Rebillon | 25 Sept | 3 Oct | 821 | 18 Oct | 20 Oct | 409 | 498 | *b* |
| | | Rp 7 Ensa | 8 Oct | 20 Oct | 563 | 18 Oct | 27 Oct | 1057 | 111 | *c* |
| | | Rp 2 Tilloy | 4 Oct | 19 Oct | 505 | 22 Oct | 7 Nov | 319 | 2093 | *e* |
| | | Rp 3 Tilloy | 4 Oct | 17 Oct | 882 | 18 Oct | 3 Nov | 653 | 2860 | *e* |
| | 3 | Rp 4 Tilloy | 2 Oct | 10 Oct | 556 | 25 Oct | 3 Nov | 283 | 2354 | *e* |
| | | Rp 6 Tilloy | 4 Oct | 20 Oct | 576 | 29 Oct | 10 Nov | 620 | 3036 | *e* |
| | | Rp 9 Tilloy | 2 Oct | 20 Oct | 391 | 22 Oct | 7 Nov | 247 | 2173 | *e* |
| | | Rp K 93 Rennes | 28 Nov | 28 Nov | 58 | 20 Oct | 22 Nov | 49 | 2913 | *f* |
| | | Rp 15 Rennes | 28 Nov | 28 Nov | 22 | 22 Nov | 22 Nov | 8 | 2329 | *f* |
| | 4 | Rp 22 Rennes | – | – | 0 | 28 Nov | 28 Nov | 1 | 2905 | *f* |
| | | Rp 26 Rennes | – | – | 0 | – | – | 0 | 3002 | *f* |
| | | Rp 32 Rennes | – | – | 0 | – | – | 0 | 4168 | *f* |

Variability in clonal responses between experimental conditions

First gynoparae were produced one week earlier in continental conditions than in oceanic conditions (dg1 = September 29th and dg1 = October 6th). The difference was even more important for dg5 which was reached two weeks earlier in continental than in oceanic conditions. No significant difference in total number of gynoparae was found between the two conditions. On the other hand, males were produced at the same date in both conditions but at a lower rate in the continental regime. The number of winged virginoparae was also lower in continental (vtot = 1084 individuals) than in oceanic conditions (vtot = 1613).

The analysis of variance performed for testing the hypothesis of a geographic adaptation among cyclical parthenogens (clones of group 1 and 2) showed neither a significant effect of geographic origin nor interaction between origin and experimental condition.

Transition from parthenogenetic to sexual reproduction

The classification following the principal component analysis of the seven variables showed six types of sequences of sexual morph production (Fig. 2, Table 3). Sequences of type *a*, *b* and *c* were characterized by a complete transition from asexual to sexual reproduction. Sequences *a* and *b* differed mainly by the timing of gynoparae production and the number of males. Type *a* was composed of clones of groups 1 and 2 reared in continental conditions which produced early gynoparae and a low number of males. Type *b* was composed of clones of groups 1 and 2 reared in oceanic conditions, with late gynoparae production, a large number of males and a small delay between gynoparae and male production.

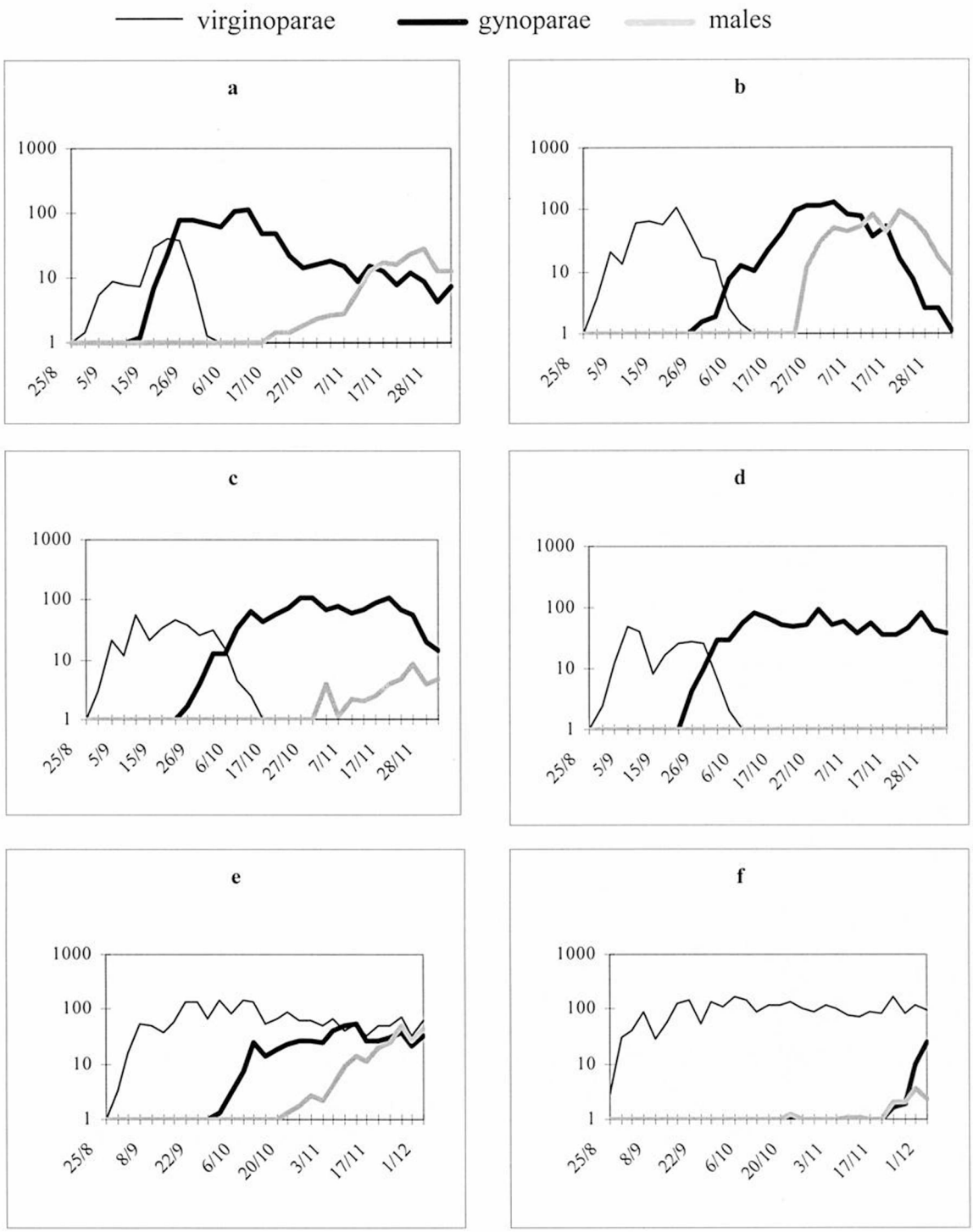


Fig. 2. Types of sequences of sexual morph production in *Rhopalosiphum padi* clones following Principal Component Analysis. Types a to f correspond to those of Table 3. Number of individuals in logarithmic scale.

Type *c* included responses of clones of groups 1 and 2 but reared in both conditions. Clones of that type produced gynoparae late and a few males. Type *d* was characterized by a continuous production of gynoparae and absence of males. Only two clones (Rp 18 Arbo and Rp 15 Colmar reared in continental conditions) belonged to this type. Type *e* was composed of clones producing asexual and sexual morphs at the same time. This incomplete transition between the two modes of reproduction corresponded to a mixed strategy with both virginoparae and sexuals produced at the same time. This type included all clones of group 3, originating from northern France. The last type, *f*, corresponded to the response of androcyclic clones (group 4) with a continuous production of winged virginoparae, a weak production of males from the middle of October and a surprising but weak and late production of gynoparae for two clones (Rp K 93 Rennes and Rp 15 Rennes).

TABLE 4. Comparison of sex ratio of *Rhopalosiphum padi* (gynoparae / [males + gynoparae]) in reference standard conditions (Rispe et al., 1999) and after exposure to conditions mimicking continental and oceanic climates.

| Group | Clones | Standardised condition | Continental condition | Oceanic condition |
|---|---|---|---|---|
| 1 | Rp10 Colmar | 0.30 | 0.94 | 0.55 |
| | Rp4 Colmar | 0.35 | 1.00 | 0.98 |
| | Rp3 Colmar | 0.40 | 0.96 | 0.52 |
| | Rp1 Colmar | 0.48 | 0.94 | 0.75 |
| | Rp15 Colmar | 0.92 | 1.00 | 1.00 |
| 2 | Rp7 Ensa | 0.27 | 0.85 | 0.35 |
| | Rp1 Arbo | 0.43 | 0.85 | 0.35 |
| | Rp1 Rebillon | 0.48 | 0.80 | 0.67 |
| | Rp8 Arbo | 0.72 | 0.78 | 0.70 |
| | Rp18 Arbo | 0.93 | 1.00 | 0.99 |
| 3 | Rp2 Tilloy | – | 0.95 | 0.61 |
| | Rp3 Tilloy | – | 0.77 | 0.57 |
| | Rp4 Tilloy | – | 0.98 | 0.66 |
| | Rp6 Tilloy | – | 0.88 | 0.48 |
| | Rp9 Tilloy | – | 0.96 | 0.61 |

Sex ratio

The variability in sex ratio obtained with reference standard conditions was high after oceanic condition exposure (range between 0.35 and 1.00) but was dramatically reduced in continental conditions (range between 0.77 and 1.00) because of the lower number of males (Table 4). Furthermore, values in reference standard conditions and oceanic conditions were significantly correlated despite the fact that Rp 4 Colmar produced very few males ($r = 0.68$ with Rp 4 Colmar and $r = 0.88$ without Rp 4 Colmar).

### Mean conditions of temperature and photoperiod for sexual morphs production

Group 4 was excluded of this analysis because sexual morphs were produced very late. First gynoparae were observed in simulated condition starting from September 29th (22°C as day temperature (d-T), 12°C as night temperature (n-T)) in oceanic conditions and from September 20th (19°C d-T, 9°C n-T) in continental conditions (Table 5). First males were detected on October 22nd (18°C d-T, 9°C n-T) in oceanic conditions and on October 23rd (11°C d-T, 3°C n-T) in continental conditions. Corresponding photophases were 11:44 in oceanic conditions and 12:15 in continental conditions for gynoparae and 10:25 in oceanic condition and 10:22 in continental conditions for the males. Gynoparae were therefore observed earlier in continental than in oceanic conditions but not males. Taking into account the date of larviposition which compensates for the time difference between the two conditions, a time lag of two weeks between gynoparae and males was observed in both conditions. Temperatures corresponding to the birth of gynoparae and males were then higher in oceanic conditions than in continental conditions. Similarly, the corresponding photophase was shorter under oceanic than continental conditions.

### Comparison with sampling data

The first appearance of males ranged from August 12th to September 24th in the eastern continental region (Colmar suction trap), from August 17th to October 14th in the western oceanic region (Rennes suction trap) and similarly from August 11th to October 8th in northern oceanic region (Arras-Loos suction trap) (Table 6). In all sites, these dates were earlier than experimental results: the mean date of first appearance was seven weeks earlier in the Colmar suction trap than in continental conditions and four weeks earlier in the Rennes suction trap than in oceanic conditions. The comparison based on corresponding larviposition dates reduced this time lag to four weeks in continental conditions and three weeks in oceanic conditions.

## DISCUSSION

### Sequences of sexual morph production

The same order of progeny sequence (parthenogenetic females-gynoparae-males) was achieved by all cyclical parthenogenetic clones. This order was similar to a pattern already described for cyclical parthenogenetic clones of *R. padi* but obtained in constant short days and low temperature (Dixon & Glen, 1971; Simon et al., 1991). Two clones out of the five previously characterized as androcyclic by Simon et al. (1991) produced gynoparae towards the very end of the simulated season. In this case, gynoparae were produced when the photophase came down below 10 h and day mean temperature was below 10°C in oceanic conditions or 5°C in continental conditions, i.e. later than most males of the other clones. The probability for the progeny of these gynoparae to take

TABLE 5. Temperatures and photophase observed at the date of the first sexual morph (a) and at calculated date of larviposition (b). Data included groups 1 to 3.

| a) | | Continental condition | | | | Oceanic condition | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Date | Day °C | Night °C | Photophase | Date | Day °C | Night °C | Photophase |
| Gynoparae | mean | 20 Sept | 19 | 9 | 12:15 | 29 Sept | 22 | 12 | 11:44 |
| | (s.d.) | (8 days) | (1.8) | (1.0) | (00:25) | (5 days) | (0.7) | (0.5) | (00:16) |
| Males | mean | 23 Oct | 11 | 3 | 10:22 | 22 Oct | 18 | 9 | 10:25 |
| | (s.d.) | (13 days) | (2.6) | (1.0) | (00:35) | (7 days) | (1.1) | (0.8) | (00:20) |
| b) | | Continental condition | | | | Oceanic condition | | | |
| | | Date | Day °C | Night °C | Photophase | Date | Day °C | Night °C | Photophase |
| Gynoparae | mean | 05 Sept | 20 | 10 | 13:06 | 17 Sept | 24 | 13 | 12:25 |
| | (s.d.) | (6 days) | (0.5) | (0.6) | (00:18) | (5 days) | (0.9) | (0.5) | (00:14) |
| Males | mean | 21 Sept | 19 | 9 | 12:11 | 05 Oct | 20 | 11 | 11:23 |
| | (s.d.) | (3 days) | (0.9) | (0.5) | (00:08) | (5 days) | (0.8) | (0.3) | (00:15) |

TABLE 6. Dates of the first males caught in suction trap from 1978 to 1995 compared to experimental results.

| | Earliest first catch | Latest first catch | Mean first catch | Mean larviposition date |
|---|---|---|---|---|
| Colmar | 12 Aug | 24 Sept | 4 Sept | 25 Aug |
| Rennes | 17 Aug | 14 Oct | 23 Sept | 13 Sept |
| Arras-Loos | 11 Aug | 8 Oct | 11 Sept | 2 Sept |
| Continental condition | 16 Oct | 21 Nov | 23 Oct | 21 Sept |
| Oceanic condition | 18 Oct | 12 Nov | 22 Oct | 5 Oct |

part in the sexual reproduction would be therefore very low to nil. Furthermore, these androcyclic clones produced only a few males. If this pattern is similarly expressed in natural conditions, this would reduce the likelihood for these clones to mate with sexual females. This is in agreement with a very limited gene flow between these two lineages as indicated by molecular studies (Simon et al., 1996a; Martinez-Torres et al., 1997). Nevertheless, the existence of such clones weakens the border between the different reproduction strategies.

No geographic adaptation in the timing of sexual morph production was found among cyclical parthenogenetic clones. However, these clones were all collected at the same latitude contrary to the work of Austin et al. (1996) showing an effect of latitude on the progeny sequences of *R. padi* clones from different sites in Great Britain, and the experiments of Lushai et al. (1996) who showed an effect of latitude on photoperiodic responses for sexual morph production.

Clones collected in northern France produced sexual morphs along with a continuous production of parthenogenetic females. Gynoparae and males of these clones were produced at the same time as those of cyclical parthenogens and can therefore potentially take part in sexual reproduction. This is the first observation of this particular mixed strategy in *R. padi* but this life-cycle variant has already been described for non host-alternating species such as *A. pisum* (MacKay, 1989), *Megoura viciae* Buckton (Lees, 1959) or *Sitobion avenae* (F.) (Dedryver et al., 1998). This mixed strategy could be an adaptive response to environmental conditions because it would allow clones to produce both migrant parthenogenetic females and gynoparae, thereby increasing their chance of survival. This mixed strategy could be particularly favoured in regions where winter climate is not predictable, as suggested by theoretical (Rispe et al., 1998a) and field data (Dedryver et al., 1998; Simon et al., 1999). Individuals collected on secondary hosts and carrying haplotype II which were at the origin of the clones of group 3, probably arose from the parthenogenetic part of clones having this mixed strategy of reproduction. Such clones are likely to be more often selected in northern France than in other regions because of irregularly cold winters (Martinez-Torres et al., 1997).

In *R. padi*, a different mixed strategy was found involving gynoparae that produced both sexual (oviparae) and parthenogenetic females (Tatchell & Parker, 1990; Simon et al., 1991). However, it is not known whether these gynoparae could reproduce partially on Poaceae and then return to bird-cherry to produce sexual females.

For most cyclical parthenogenetic clones, sex-ratio variation among clones previously detected in reference standard conditions was maintained in oceanic conditions. This result suggests that sex-ratio determinism has probably an important genetic component. This hypothesis of a genetic basis for sex allocation is supported by crossing experiments which are currently undertaken (Rispe et al., 1999). Sex allocation in nature can vary with environmental conditions. For instance, the percentage of *R. padi* males in British suction traps was positively correlated with temperature of the end of summer and beginning of autumn (Rispe et al., 1998b). Since males are produced after gynoparae, the unexpected earlier mortality of parents may explain a deficit in males. This might have been the case in our experiment because sex-ratios were female-biased in continental conditions where the day temperatures were 7°C lower than in oceanic conditions, suggesting that complete sequences could not be achieved in a colder climate.

**Timing of sexual morph production**

In our experiments, the photophase decrease was the same in both oceanic and continental conditions. There was only a difference in temperature which was lower and decreased more rapidly in continental than in oceanic conditions. Considering the larviposition date, gynoparae were produced earlier in continental conditions at a mean day temperature of 15°C and a photophase of 13:06, and later in oceanic conditions at a mean day temperature of 18°C and a photophase of 12:25. These results suggest that sexuals production depends on a combination of both factors which varies with geographic location. In this way and as long as temperature is high enough, aphids should sustain parthenogenetic reproduction. This strategy represents a selective advantage because it increases the fitness of clones. The reasoning was here applied to the larviposition date of gynoparae as they should precede males to maximise the success of mating (Ward & Wellings, 1994). It could be applied just as well to the males and probably to previous generations because parents are sensitive to photoperiod during the beginning of their nymphal life (Dixon & Dewar, 1974).

The hypothesis of a timer based on a photoperiodic clock and potentially regulated by temperature has already been invoked in the induction of sexual reproduction in *M. viciae*, *A. pisum* (Lees, 1960; Lees, 1990), *M. persicae* (Blackman, 1975) and *Aphis fabae* (Hardie, 1987; Vaz Nunes & Hardie, 1992). A two way determination of sexual morph production was already described in *R. padi* and other species like *A. fabae* or *D. plantaginea* (Dixon & Glen, 1971). For *R. padi*, a complete transition from asexual to sexual morphs production was achieved between 14 h and 15 h of photophase at 10°C, or between 12 h and 13 h at 14°C. It could be nevertheless experimentally difficult to separate the action of these two factors on sexual morph determination (Leather et al., 1993). These two factors act in a parallel way on aphids and on

their host plant as well. Leaf senescence, which represents a biological barrier for aphid reproduction, can be postponed if high temperatures persist in the autumn (Ward et al., 1984).

These results underline the need for a better understanding of the influence of the whole array of environmental factors inducing the transition from parthenogenetic to sexual reproduction in aphids.

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