Bulletin of Entomological Research (2015) **105**, 694–703
doi:10.1017/S0007485315000619

Longitudinal clines in the frequency distribution of 'super-clones' in an aphid crop pest

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Abstract

Parthenogenesis is the main mode of reproduction of aphids. Their populations are therefore composed of clones whose frequency distribution varies in space and time. Previous population genetic studies on aphids have highlighted the existence of highly abundant clones ('super-clones'), distributed over large geographic areas and persisting over time. Whether the abundance of 'super-clones' results from their ecological success or from stochastic forces, such as drift and migration, is an open question. Here, we looked for the existence of clines in clonal frequency along a climatic gradient in the cereal aphid *Rhopalosiphum padi* (Linnaeus, 1758) and examined the possible influence of geographical distance and environmental variables in the buildup and maintenance of such clonal clines. We investigated the spatial distribution of the commonest genotypes of *R. padi* by sampling populations along an east–west transect in maize fields in the northern half of France in both spring and late summer. Individual aphids were genotyped at several polymorphic loci, allowing the assessment of frequency distributions of multilocus genotypes (MLGs) across the cropping season. We found several MLGs showing longitudinal clines in their frequency distribution in both spring and summer. In particular, two dominant asexual genotypes of *R. padi* showed inverted geographical clines, which could suggest divergent adaptations to environmental conditions. We concluded that while the distribution of some 'super-clones' of *R. padi* seems most likely driven by the action of migration and genetic drift, selection could be also involved in the establishment of longitudinal clines of others.

Keywords: aphids, genotypic diversity, selection, drift, cyclical parthenogenesis

(Accepted 3 July 2015; First published online 17 August 2015)

Introduction

While sexual reproduction is widespread among metazoans, some groups of organisms use alternative reproductive modes during all or part of their life cycle. Aphids typically reproduce by cyclical parthenogenesis whereby a round of several clonal (apomictic) generations is followed by a single sexual generation to complete the annual life-cycle. In addition, many aphid species show a coexistence of cyclically (hereafter termed 'sexual') and obligatory (hereafter termed 'asexual') parthenogenetic lineages: sexual lineages shift from clonal to sexual reproduction once a year, while asexual lineages reproduce by parthenogenesis all year round. Some

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asexual lineages are yet able to produce both asexual and sexual forms in autumn and to contribute to sexual reproduction (Halkett *et al*., 2005, 2008). Sexual lineages of aphids are favored in regions with harsh winter because they can survive as cold-resistant eggs, while frost-susceptible asexual lineages are favored in regions with mild winter because they remain reproductively active all year round. In regions with fluctuating winter conditions, aphid populations encompass a mixture of sexual and asexual lineages. Winter conditions are thus an important selective factor that determines the local persistence of asexual lineages and shapes the spatiotemporal distribution of the two types of lineages (Simon *et al*., 2002). Previous genetic studies on aphid populations sampled in areas allowing parthenogenetic overwintering have reported, in many species, the existence of highly abundant clones ('super-clones') distributed over large geographic scales and persisting through time (Vorburger *et al*., 2003a; Gilabert *et al*., 2009; Piffaretti *et al*., 2013). The occurrence of such 'super-clones' raises questions about the forces responsible for their predominance (Vorburger *et al*., 2003b; Castañeda *et al*., 2010; Fenton *et al*., 2010). Are they abundant because of their ecological success or because of stochastic forces? It has been indeed shown that abundant clones can also arise by drift and be maintained by chance only due to extreme clonal lifespan and size (Arnaud-Haond *et al*., 2012). Moreover, migration is known to be intense in aphid species because of their ability to produce massive numbers of winged individuals that disperse widely (Loxdale & Lushai, 2007). Hence, selection exerted on these clonal populations has to oppose to strong homogenizing and randomizing forces. Rapid and sometimes drastic fluctuations in clonal frequencies over time and/or space are also found in other groups of parthenogenetic organisms (e.g. Weeks & Hoffmann, 1998; Niklasson *et al*., 2004; Vorburger, 2006; Allen & Lynch, 2012), and are usually interpreted as resulting from either a change in selective forces or random process. In this study, we investigated clines in clonal frequency along a climatic gradient in the bird-cherry oat aphid *Rhopalosiphum padi* (Linnaeus, 1758), and explored the possible role of geographical distance and environmental variables on the establishment of clonal clines. Clines in clonal frequencies can be produced by drift and restricted gene flow (i.e. through isolation by distance; Vasemagi, 2006), though these neutral clines would not be expected to be stable with time (Rieux *et al*., 2013). The observation of replicated and/or independent clines along climatic gradients could provide support for the local selection (e.g. Paaby *et al*., 2010; Samis *et al*., 2012) of aphid genotypes with different thermal optima.

The two types of lineages with distinct reproductive modes exist in *R. padi*, and can even co-exist in local populations (Gilabert *et al*., 2009, 2014). Sexual lineages of *R. padi* reproduce from spring to autumn by parthenogenesis on Poaceae plants and switch in autumn to their winter host, the bird-cherry tree *Prunus padus*, where the sexual reproduction takes place. By contrast, asexual lineages reproduce parthenogenetically all year round on Poaceae hosts. Earlier works using microsatellite markers highlighted the occurrence of highly represented clones in populations of *R. padi* in areas with mild winters (Halkett *et al*., 2005; Gilabert *et al*., 2009). We postulated that these 'super-clones' may have been selected by agricultural practices or other environmental factors (Gilabert *et al*., 2009), an explanation that needs to be reconsidered with alternative hypotheses presented above.

Here, we first determined the spatial distribution of clonal lines of *R. padi* characterized using highly polymorphic markers, along a 600 km transect through Northern France, at two time points. Because the climate conditions are expected to be the main effect constraining the survival of aphid asexual lineages, the sampling scheme was designed to fit a climatic gradient from oceanic conditions in the west with mild winters, to continental conditions in the east with cold winters. We then looked for the existence of clines in clonal frequencies along this climatic gradient by exploring changes in genotypic structure over a season, as well as across years on a restricted set of populations. We finally asked if those clines of clonal lines may be explained by stochastic forces (some variations of clonal frequencies due to genetic drift) or by deterministic ones like climatic variables.

Materials and methods

Aphid collections

Aphids were mostly collected in 2007 along an east–west transect in the northern half of France at two occasions, in spring and at the end of summer. In the northern half of France, climate shows an environmental gradient from an oceanic climate in the western part characterized by mild temperatures and high rainfall, to a continental one in the East characterized by warm summers and cold winters with relatively important snowfall and high number of frost days (see fig. S1). Consequently, temperatures vary continuously along the transect, as illustrated with the mean recorded minimal and maximal temperatures for the first 4 months of the year 2007 (fig. S2).To reduce the possible influence of host plant on patterns of genetic differentiation, aphids were only collected from maize fields. In spring 2007, we sampled 18 fields of maize (fig. 1), approximately 50 km apart from each other. The spring sample consisted of winged immigrant aphids collected by late spring (from the 29th of May to the 7th of June; table S1), just after the migration: (i) of sexual lineages from the winter host *P. padus* to the summer Poaceae hosts, and (ii) of asexual lineages from their overwintering sites (young cereals, wild Poaceae) to summer Poaceae hosts (Dedryver, 1983; Simon *et al*., 1991). The second sampling was carried out in late summer and early autumn (between the 23rd of August and the 3rd of October; table S1), before the migration: (i) of sexual lineages back to *P. padus* and (ii) of asexual lineages to their Poaceae overwintering hosts. It was not possible to conduct the summer sampling in the same fields sampled in spring; summer fields were thus sampled along the same environmental transect but between 11 and 38 km away from a closest spring field. Not all spring sampled fields could have a geographically close field sampled in summer so that the summer sampling included 14 maize fields (fig. 1). Hereafter, a 'site' refers to a pair of nearby maize fields (one sampled in spring and its closest field sampled in summer), thus defining 13 sites (see fig. 1). Only wingless individuals were collected in the second survey in order to ensure that sampled aphids were born and developed on maize. Only one individual was collected per plant and plants of collection were separated by at least 1 m to avoid multiple sampling of the same colony. Aphids were stored in alcohol at −20°C before the analysis. The number of aphids per field ranged from 24 to 76 in spring (mean ± SD = 48 ± 13.43; $N = 864$) and from 16 to 45 in summer (mean ± SD = 31.14 ± 9.05; $N = 436$). Aphids collected on maize fields in autumn 2005 in Le Rheu and around Reims ($N = 209$) and in autumn 2006 in Chizé, Pleine-Fougères and Saint-Hilaire-en-Woëvre ($N = 189$) (fig. 1) were added to

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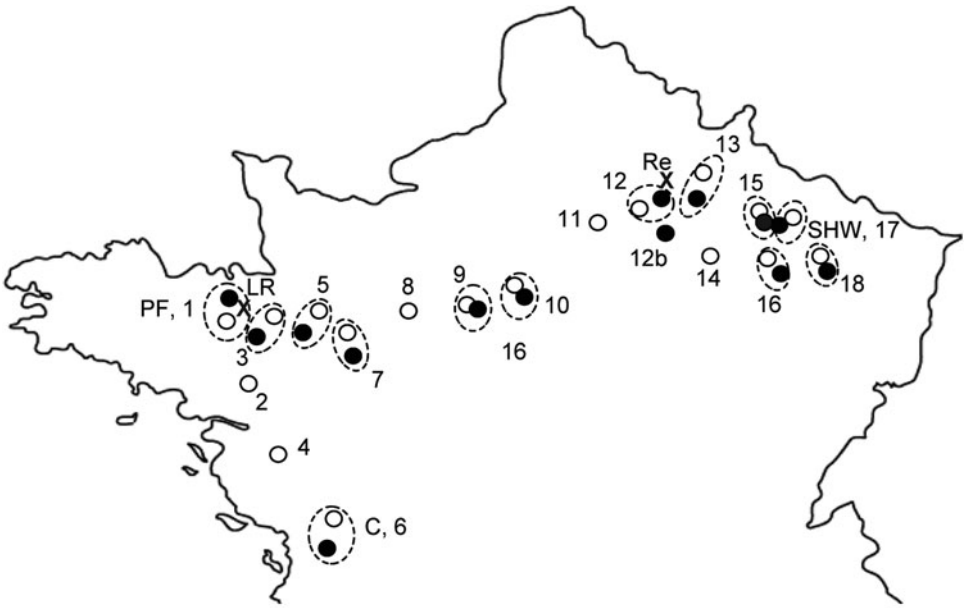

	SP01	SP06	SP10	SP13
SP01				
SP06	237			
SP10	287	305		
SP13	489	517	218	
SP18	586	559	300	144

Fig. 1. Locations of the sampled fields. Fields sampled in spring 2007 are represented by white circles, those sampled at the end of the summer by black circles. Populations are numbered from west to east; each summer population has the same number as its closest spring population (except for populations 2, 4, 8, 11 and 14 because no fields were sampled in summer, and for population 12b, which has been sampled in summer only). Aphids sampled on maize in autumn 2005 were collected in Le Rheu (LR) and around Reims (Re) (black crosses); those sampled in autumn 2006 were collected in Chizé (C), Pleine-Fougères (PF) and Saint-Hilaire-en-Woëvre (SHW) (corresponding to 2007 fields number 6, 1 and 17, respectively). Distance (in km) between a few fields sampled in the spring is given.

determine whether the commonest multilocus genotypes (MLGs) detected in spring and summer 2007 had previously been detected at both extremes of the transect. In the following text, a population refers to one sampling field sampled at one specific date. This study therefore includes 37 populations: 2 sampled in autumn 2005, 3 in autumn 2006, 18 in spring 2007 and 14 in summer 2007.

Assessment of genotypic diversity

Genetic diversity and structure were assessed by analyzing eight polymorphic microsatellite loci. DNA extraction from individual aphids and genotyping at the eight loci were performed as described in Gilabert *et al.* (2009).

To evaluate a possible lack of resolution of our markers, we estimated the probability that two individuals could by chance share the same MLG though being produced by sexual reproduction following Waits *et al.* (2001). Two estimators were computed under the hypotheses of extreme reproduction regimes to provide the lower and the upper bounds of the actual value: $P_{(ID)\ unbiased}$ under Hardy–Weinberg expectations and $P_{(ID)\ sibs}$ under strong inbreeding. Genotypic diversity was estimated as the ratio G/N, with G being the number of discriminated MLGs in the sample, and N the number of individuals. Index of genotypic diversity was correlated with sample size (Spearman correlation; $\rho = 0.57$; $P < 0.001$). Therefore, for comparison, we estimated genotypic diversity in all samples by resampling 19 individuals 1000 times (3 fields with less than 19 individuals were not considered) and then using the average (±SE) over the 1000 replications (Arnaud-Haond & Belkhir, 2007; available at http://www.ccmar.ualg.pt/maree/software.php?soft=genclon). Clonal diversity (CD), less sensitive to sample size than genotypic diversity, was also estimated using the inverse of Simpson index ($CD = 1/\sum pi^2$, with pi being the frequency of genotype i in the population). This measure accounts for both evenness and diversity, and ranges between one, when all individuals share the same genotype, and G, when all the genotypes are present in equal frequencies. For these analyses of clonal diversity, we considered only individuals without any missing data to assign all individuals unambiguously to a given MLG ($N = 765$ and 297 in spring and summer, respectively). Calculations were performed using the software GenClone v2.0 (Arnaud-Haond & Belkhir, 2007).

Previous studies showed that the Bayesian clustering method implemented in Structure (Pritchard *et al.*, 2000) allowed to unambiguously and reliably assign the reproductive mode of *R. padi* genotypes, the sexual and asexual lineages

https://doi.org/10.1017/S0007485315000619 Published online by Cambridge University Press

being separated in two genetic clusters (Halkett *et al*., 2005, 2008; Gilabert *et al*., 2009). We therefore used that approach to assign the putative reproductive mode of each aphid genotype. Runs were performed without multicopies and with a reference sample of sexual and asexual genotypes (see Gilabert *et al*. (2009) for more details) to check for the reliability of reproductive mode assignation. Five independent runs assuming an admixture model and with a burn-in period of 20,000 iterations followed by 200,000 iterations were performed to assess the consistency of the results. A factorial correspondence analysis (FCA) was also performed on the MLGs without missing data using Genetix (Belkhir *et al*., 2004) to investigate the genetic relationships between the different lineages with an approach that does not rely on population model assumptions.

Spatial and temporal genetic structure

Population structure was explored assuming *a priori* grouping of individuals according to the sampled field and the period of collection. We examined changes in clonal composition along transects and between spring and summer. First, relationships between genotypic diversity in subsamples of 19 individuals (G_{19}/N) and CD and longitude and/or sampling period were tested by fitting linear models (LM, using normal errors). Normality of residuals was checked and confirmed using Shapiro–Wilk test of normality and a quantile–quantile plot to compare the distribution of the residuals of our models to normal ones. We then explored the spatial and temporal changes in MLG composition of the asexual populations. Proportions of each MLG represented by at least 20 individuals in the entire collection (i.e. representing more than 1.5% of the total sampling) were fitted by generalized linear models (GLM using a binomial distribution and a logit link) with the following factors: longitude, sampling period and their interaction. Because two MLGs (*G*1 and *G*2) were overrepresented in all sampled fields and may hide some patterns of genetic structure, we estimated the proportions of the other four most common asexual MLGs by excluding *G*1 and *G*2. Proportions were estimated considering asexual genotypes only. Six fields had less than five individuals after the exclusion of the sexual MLGs, the MLGs not assigned to any genetic cluster, and the two commonest MLGs, *G*1 and *G*2. They were therefore not considered in the analyses of the four abovementioned common MLGs. We did not explore the influence of latitude because of the narrow considered latitude range, and because longitude and latitude were correlated in the transect. Because climate can affect aphid's biology and growth to different extent depending on the lineage (Rispe *et al*., 1996), we investigated the influence of some climatic variables on the distribution of the commonest MLGs. In Europe, monthly climatic variables are often correlated with longitude and between themselves (see, for example, the correlations between the longitude and climatic variables for the months of January, February, March and April in fig. S2). It is thus difficult to highlight a particular important variable or a particular time window influencing a trait. Consequently, the mean monthly minimal temperature of February (min *T*.), which is a good predictor of aphid population growth in spring (Vialatte *et al*., 2005), the mean monthly maximal temperature (max *T*.), the precipitation height (Prec.), and the duration of sunshine period (sunshine) in February were chosen to encapsulate the influence of the climate on the proportion of the most common MLGs in spring and summer. Because of the correlations between the different variables, we did not include all of them in a single model and tested for the influence of each climatic variable separately in a single-variable model and compared them to the 'geographic' model (i.e. the model that included the longitude) using the Akaike Information Criterion (AIC). For each model, the significance of the variable was tested by means of an analysis of deviance using a likelihood ratio test. Two independent models were performed according to the period of sampling, except for the MLG *G*4 for which we detected two individuals only in summer. Two fields sampled in spring (SP03 and SP05) and one in summer (SU05) were not included in the analyses of the climatic influence because of missing climatic variables. The climatic data were obtained from the French meteorological service (http://www.meteofrance.fr/). We used for each field the data from the closest meteorological station.

Models were fitted using R version 2.3.1 (R Development Core Team, 2012).

Results

Overall diversity

The probability that two identical MLGs at the eight microsatellites resulted from sexual reproduction was low, between 5.55×10^{-6} ($P_{(ID)\ unbiased}$) and 4.37×10^{-3} ($P_{(ID)\ sibs}$), indicating that the resolution of our markers was sufficient to assume confidently that two individuals sharing the same MLG belonged to the same clone (see Waits *et al*., 2001). Among the 1300 aphids collected on maize in 2007, we identified 206 MLGs ($G/N \approx 0.158$), 48 of those being shared by at least two individuals. Two MLGs (*G*1 and *G*2) were highly dominant and accounted for 41.4 and 18.5%, respectively, of the total sample collected in 2007 on maize. These MLGs were identical to the previously reported *G*1 and *G*2 MLGs in French populations in autumn 2005 and 2006, and which have likely persisted for at least 10 years since they have been detected in western France between 1998 and 2001 (Gilabert *et al*., 2009). In autumn 2005 and 2006, genotype *G*1 accounted for 22% (14.46% in Le Rheu and 26.98% around Reims) and 58.7% (81.2% in Chizé, 51.5% in Pleine-Fougères and 38.5% in Saint-Hilaire-en-Woëvre) of all the aphids sampled on maize, respectively. Genotype *G*2 accounted for 37.3% in 2005 (65.06% in Le Rheu and 19.05% around Reims) and 5.3% in 2006 (5.8% in Chizé, 8.8% in Pleine-Fougères and 0% in Saint-Hilaire-en-Woëvre; see fig. 2). Other MLGs individually accounted for <5% of the total sample.

We successfully assigned the reproductive mode of *R. padi* genotypes using the Bayesian clustering approach ran for $k = 2$. Considering a threshold of $q > 0.8$, 71% of MLGs (146/206) representing ~94% of the individuals (1219/1300) were assigned to either cluster. Less than 8% (93 individuals) of the assigned individuals were assigned to the sexual cluster; the other ~92% (1126) belonged to the asexual cluster. The asexual cluster accounted for ~89% (730/817) and ~99% (396/402) of the individuals sampled in spring and summer respectively, and ~72% (67/93) and ~92% (49/53) of the MLGs identified in spring and summer, respectively. As expected, the majority of repeated MLGs (36/41 assigned repeated MLGs) was assigned to the asexual cluster whereas the sexual cluster mainly contained unique MLGs. The FCA indicated that the lineages were not differentiated, with the two factorial components each explaining respectively 4.13 and 3.28% of the genetic variability (fig. S3).

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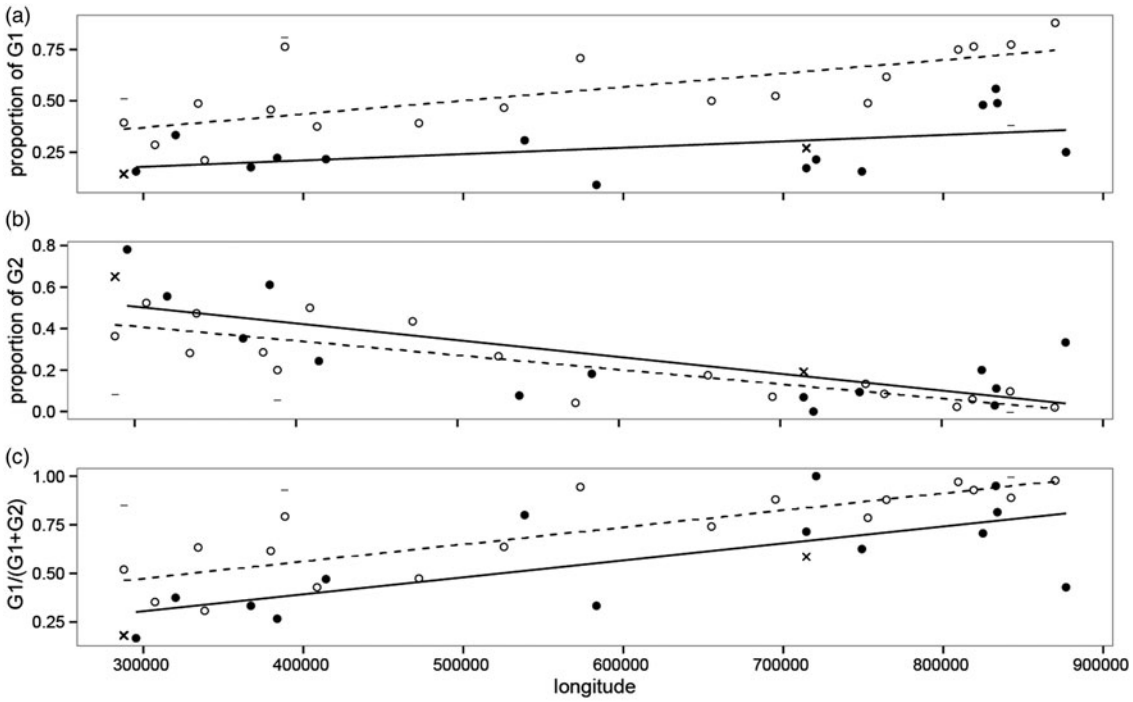

Fig. 2. Distribution of the proportions of the two commonest genotypes (*G*1 – (a); *G*2 – (b)) in each population according to longitude in spring 2007 (white circles and dashed line), in summer 2007 (black circles and continuous line), and in the three areas sampled in autumn 2006 (black underscores) and in the two areas sampled in autumn 2005 (black crosses). Relative proportion of *G*1 compared with *G*1 + *G*2 is also given according to longitude (c).

Genotypic diversity was 0.15 ($G = 131$) in spring and 0.22 ($G = 98$) in summer when considering all the sampled individuals, and 0.14 ($G = 106$) and 0.22 ($G = 64$) in spring and late summer respectively when removing individuals with missing data. When estimated by resampling (19 individuals 1000 times) to correct for sample size, genotypic diversity ranged between 0.13 (sample SP06) and 0.53 (sample SP03) in spring (mean ± SD = 0.36 ± 0.11) and between 0.19 (samples SU01 and SU06) and 0.66 (sample SU13) at the end of the summer (mean ± SD = 0.39 ± 0.16) (table 1). CD ranged from 1.41 (sample SP18) to 5.43 (sample SP01) in spring (mean ± SD = 3.12 ± 1.15) and between 1.77 (sample SU01) and 11.36 (sample SU13) in late summer (mean ± SD = 5.37 ± .3.14). Analyses of variance indicated that neither longitude ($F_{(1,\ 26)} = 0.07$; $P = 0.8$) nor sampling period ($F_{(1,\ 26)} = 0.49$; $P = 0.5$) influenced genotypic diversity, and that sampling period only contributed to clonal diversity, which was higher in summer (sampling period: $F_{(1,\ 29)} = 7.96$; $P = 0.009$; longitude: $F_{(1,\ 29)} = 0.11$; $P = 0.74$).

Spatial and temporal changes in clonal composition

Asexual populations differed in their clonal composition along the transect and between spring and summer 2007 as suggested by changes in MLGs frequency distribution. In particular, the proportion of *G*1 increased from west to east when that of *G*2 decreased, both in spring and in late summer (table 2; figs 2a, b). Proportions of *G*1 were higher in spring than in summer in 12 sites (over 13 sampled in both spring and summer), while *G*2 proportions increased between spring and summer in eight of the 13 sites sampled at the two occasions. The same pattern was observed when considering the two dominant genotypes *G*1 and *G*2 only (table 2; fig. 2c). In 2005 and 2006, only two and three observations were available, respectively, preventing any statistical analysis. However, patterns observed in 2005 were consistent with those in 2007 (fig. 2) suggesting that clines in frequencies of *G*1 and *G*2 may have been established before 2007.

The influence of longitude and sampling period were also tested on the four other most common MLGs, after removing *G*1 and *G*2 from the samples (table S2). For three of them (*G*3, *G*10 and *G*11), the interaction between longitude and the sampling period was significant (table S2). Their proportion varied significantly with the longitude in summer only (fig. S4). The last genotype was significantly more abundant in spring than in summer but did not vary with the longitude (table S2; fig. S4). Genotype *G*4 was not detected in 2005 on maize (although it was found on wheat regrowths around Reims; A. Gilabert, Personal Observation) but was observed in Pleine-Fougères in 2006 (4.4% of the individuals). Genotype *G*10 was detected in both 2005 (34.9% of the individuals collected in Le Rheu and 14.3% around Reims) and 2006 (1.5% in Pleine-Fougères, 4% in Chizé and 0% in Saint-Hilaire-en-Woëvre) and genotype *G*11 in 2006 (4.4% in Pleine-Fougères, 1% in Chizé and 1.9% in Saint-Hilaire-en-Woëvre). The frequencies of genotype *G*10 in autumn 2005 were consistent with the ones observed in summer 2007, contrary to the frequencies observed in autumn 2006, which were relatively low.

Influence of geographic distance and climatic variables on the distribution of commonest genotypes

The comparison of the single-variable models indicated that the geographical model was, in spring, the best model

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Table 1. Features of spring (SP) and summer (SU) populations of *R. padi* along a west-east transect in France. Number of individuals (*N*); number of distinct MLGs identified (*G*), genotypic diversity (*G*/*N*) on each entire population and the average (±SE) of 1000 subsamples of 19 individuals (G_{19}, G_{19}/N); clonal diversity (CD, see the text). G_{19} and G_{19}/N are not available for three populations with less than 19 sampled individuals (NA). Individuals with missing data were removed for these analyses.

Sample name	*N*	*G*	*G*/*N*	$G_{19} \pm$ SE	G_{19}/N	CD
SP01	46	15	0.33	8.26 ± 0.05	0.43	5.43
SP02	50	15	0.3	7.79 ± 0.05	0.41	3.62
SP03	30	16	0.53	11.22 ± 0.04	0.59	4.02
SP04	60	13	0.22	6.5 ± 0.04	0.34	4.34
SP05	39	14	0.36	8.32 ± 0.05	0.44	3.93
SP06	45	3	0.07	2.4 ± 0.02	0.13	1.55
SP07	24	6	0.25	5.36 ± 0.02	0.28	3.65
SP08	19	6	0.32	6 ± 0.00	0.32	2.93
SP09	45	12	0.27	7.07 ± 0.04	0.37	3.68
SP10	72	16	0.22	6.17 ± 0.05	0.32	2.02
SP11	34	10	0.29	7.18 ± 0.04	0.38	3.14
SP12	49	21	0.43	10.29 ± 0.05	0.54	4.01
SP13	49	15	0.31	8.58 ± 0.04	0.45	4.16
SP14	46	14	0.3	7.67 ± 0.04	0.4	2.66
SP15	36	10	0.28	5.99 ± 0.04	0.32	1.87
SP16	54	12	0.22	5.88 ± 0.04	0.31	1.97
SP17	23	4	0.17	3.78 ± 0.01	0.2	1.75
SP18	44	8	0.18	3.99 ± 0.04	0.21	1.41
SU01	26	4	0.15	3.67 ± 0.02	0.19	1.77
SU03	19	6	0.32	6 ± 0.00	0.32	2.98
SU05	19	9	0.47	9 ± 0.00	0.47	6.12
SU06	30	4	0.13	3.61 ± 0.02	0.19	2.02
SU07	27	14	0.52	11.01 ± 0.04	0.58	8.58
SU09	23	10	0.43	9.06 ± 0.02	0.48	5.94
SU10	14	9	0.64	NA	NA	8.17
SU12	20	11	0.55	10.67 ± 0.01	0.56	8
SU12b	11	9	0.82	NA	NA	8.07
SU13	25	15	0.6	12.62 ± 0.03	0.66	11.36
SU15	22	5	0.23	4.84 ± 0.01	0.25	2.72
SU16	20	7	0.35	6.72 ± 0.01	0.35	1.98
SU17	30	7	0.23	5.6 ± 0.03	0.29	2.14
SU18	11	6	0.55	NA	NA	5.26

Table 2. Sources of variations in proportion of the two dominant genotypes among French populations of *R. padi*.

Model parameter	Deviance (%)	χ^2	d.f.	P (>χ^2)
Null GLM on proportion of *G*1				
Longitude	23.53	55.47	1	***
Sampling period	43.26	101.98	1	***
Longitude × sampling period	0.1	0.265	1	ns
Null GLM on proportion of *G*2			1	
Longitude	63.87	152.93	1	***
Sampling period	4.06	9.71	1	**
Longitude × sampling period	0.1	0.29	1	ns
Null GLM on proportion of *G*1 compared with *G*1 + *G*2				
Longitude	55.11	129.4	1	***
Sampling period	15.06	35.35	1	***
Longitude × sampling period	0.03	0.08	1	ns

χ^2, likelihood-ratio chi-squared.
***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns $P > 0.05$.

for the two commonest MLGs, *G*1 and *G*2 (table 3). Unlike *G*2, most of the variance of *G*1 distribution along the transect remains to be explained (table 3). For the four other common MLGs, none of the tested models explained their distribution. In summer, the climatic models performed significantly better than the geographic model to explain the distribution of four MLGs (table 3). For *G*1 and *G*10, only the model that included the variable 'min *T*.' was better than the geographic one; for *G*2 and *G*11, the models containing the variable 'max *T*.' for both MLGs and 'Prec.' for *G*11 were also better than the geographic model. By contrast, the geographic model was the best model for *G*3 (table 3).

Discussion

In this study, we confirmed previous results showing that populations of *R. padi* in Northern France are mainly composed of asexual genotypes, probably because of favorable winter climate conditions (notably in the oceanic zone) for parthenogenetic overwintering (Delmotte *et al.*, 2002; Gilabert *et al.*, 2009). We also observed, such as in many instances of parthenogenetic organisms, that *R. padi* populations consisted of a majority of rare clones and a few abundant ones. Among the common ones, five (*G*1, *G*2, *G*3, *G*10 and *G*11) were abundant, or relatively abundant, throughout the growing season (as suggested by their relative abundance in both samplings), whereas the last one (*G*4) was among the most common MLGs in spring only. More strikingly, we reported longitudinal clines in the frequency distribution of these abundant clones along a 600 km transect of maize fields in Northern France. Some of these clonal clines were observed at two occasions, at the beginning and the end of the warm season. In particular, two common MLGs distributed over the whole transect (*G*1 and *G*2) presented patterns of clinal variation, clines being consistent between spring and summer. In some instances, models containing a climatic variable appeared to perform better than the geographic one to explain the frequency distribution of MLGs, though this was not always the case (i.e. for MLGs *G*3).

Clinal patterns in the frequency distribution of MLGs along a geographical transect can arise through drift and isolation by distance (Vasemagi, 2006) or may reflect the effect of selection acting along the environmental gradient (Hoffmann & Weeks, 2007) or both. Gene flow, often occurring preferentially between neighboring populations, may result in correlations of their allele/genotype frequencies and create clinal distributions of alleles or genotypes. However, neutral clines driven by drift and migration are expected to be relatively unstable over time and/or space as drift will lead to random allele fixation or loss, leading to the random fluctuation of allele frequencies and to the fluctuation of the shape and width of the cline (Polechová & Barton, 2011). In highly migratory species, this can result in the homogenization of allelic frequencies, and in turn, the erosion of the clines. Hence, when parallel clines are observed or when clines are temporally stable, their occurrence likely involves a role of selection, together with effects of stochastic forces. The analyses of the six most common *R. padi* MLGs according to the longitude or climatic variables suggest that genetic drift plays an important part in shaping their geographic distribution, but that selection may also be involved. The presence of transient clines best explained by longitude rather than climatic variables, such as those observed in *G*3, may indicate that drift and

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Table 3. Fit of the geographic and climatic models. We highlighted in bold the best models inferred from the AIC values.

Model parameter	AIC	Deviance (%)	χ^2	d.f.	P (>χ^2)
(a) Spring					
Proportion of *G*1					
Null model	*150.29*				
Longitude	**120.85**	**37.20**	**31.45**	**1**	***
min *T.*	126.82	30.13	25.47	1	***
max *T.*	138.75	16.03	13.55	1	***
prec.	147.98	5.1	4.31	1	*
Sunshine	150.55	2.07	1.75	1	ns
Proportion of *G*2					
Null model	*160.46*				
Longitude	**90.69**	**67.96**	**71.76**	**1**	***
min *T.*	97.85	61.19	64.61	1	***
max *T.*	111.49	48.27	50.97	1	***
prec.	161.54	0.87	0.92	1	ns
Sunshine	138.16	23.01	24.3	1	***
Proportion of *G*3					
Null model	*31.06*				
Longitude	32.81	1.8	0.24	1	ns
min *T.*	33.03	0.15	0.02	1	ns
max *T.*	32.99	0.45	0.06	1	ns
prec.	31.68	10.37	1.38	1	ns
Sunshine	33.06	<0.001	<0.001	1	ns
Proportion of *G*4					
Null model	*35.69*				
Longitude	37.33	3.25	0.36	1	ns
min *T.*	37.38	2.8	0.31	1	ns
max *T.*	37.68	0.09	0.01	1	ns
prec.	37.66	0.27	0.03	1	ns
Sunshine	35.4	20.71	2.29	1	ns
Proportion of *G*10					
Null model	*29.88*				
Longitude	30.91	7.7	0.97	1	ns
min *T.*	29.24	20.87	2.63	1	ns
max *T.*	28.44	27.22	3.43	1	ns
prec.	31.85	0.16	0.02	1	ns
Sunshine	29.12	21.9	2.76	1	ns
Proportion of *G*11					
Null model	*33.82*				
Longitude	33.92	19.13	1.89	1	ns
min *T.*	33.1	27.43	2.71	1	ns
max *T.*	33.51	23.38	2.31	1	ns
prec.	35.67	1.52	0.15	1	ns
Sunshine	35.54	2.83	0.28	1	ns
(b) Summer					
Proportion of *G*1					
Null model	*84.81*				
Longitude	74.38	32.07	12.43	1	***
min *T.*	**70.57**	**41.9**	**16.24**	**1**	***
max *T.*	76.46	26.7	10.35	1	**
prec.	77.33	24.43	9.47	1	**
Sunshine	86.54	0.7	0.27	1	ns
Proportion of *G*2					
Null model	*151.92*				
Longitude	81.34	64.38	72.58	1	***
min *T.*	**79.57**	**65.95**	**74.35**	**1**	***
max *T.*	**81.07**	**64.62**	**72.85**	**1**	***
prec.	142.09	10.49	11.83	1	***
Sunshine	103.84	44.42	50.08	1	***
Proportion of *G*3					
Null model	*38.63*				
Longitude	**19.61**	**78.35**	**21.02**	**1**	***
min *T.*	24.52	60.04	16.11	1	***
max *T.*	34.02	24.64	6.61	1	*
prec.	32.95	28.62	7.68	1	**
Sunshine	39.17	5.44	1.46	1	ns
Proportion of *G*4					
NA (not enough data in summer fields)					
Proportion of *G*10					
Null model	*38.67*				
Longitude	34.93	23.62	5.74	1	*
min *T.*	**34.32**	**26.13**	**6.35**	**1**	*
max *T.*	35.7	20.45	4.97	1	*
prec.	39.67	4.12	1	1	ns
Sunshine	40.49	0.7	0.17	1	ns
Proportion of *G*11					
Null model	*54.49*				
Longitude	47.97	22.42	8.52	1	**
min *T.*	**42.81**	**36**	**13.68**	**1**	***
max *T.*	**44.8**	**30.76**	**11.69**	**1**	***
prec.	**43.87**	**33.21**	**12.62**	**1**	***
Sunshine	56.18	0.82	0.31	1	ns

χ^2, likelihood-ratio chi-squared.
***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns $P > 0.05$.

isolation by distance could be responsible for these clines. However, selection might account for the spatial distribution of other genotypes, such as the two most common ones observed in this study, which seem to be relatively temporally stable as two independent clines along the same gradient were detected at the beginning and the end of the growing season. To establish such clines of clonal frequencies by drift, populations should have experienced a strong bottleneck with two refuges at both extremities of the clines, allowing randomly sorted genotypes to increase in frequency. Then, to have those clines maintained in time between our sampling dates, this bottleneck should have been recent and dispersal should be low to allow the persistence of clines. However, migration is known to be important in aphids both in spring and summer (Hullé *et al.*, 1994; Mashanova *et al.*, 2008; Paulson *et al.*, 2009), involving either local, or more rarely, long-distance movements (Loxdale *et al.*, 1993). In addition, aphid species often experience strong population bottlenecks during the growing season (reviewed in Karley *et al.* (2004)), and colonies are characterized by low establishment rates and high extinction rates resulting in a high colony turnover (Massonnet *et al.*, 2002; Fievet *et al.*, 2007). Under predominant stochastic forces, both effects (drift and migration) should induce large variation in clonal frequencies between seasons and years, which is observed for example for genotypes *G*4. We thus hypothesize that, although the distribution of MLGs is likely shaped by migration and genetic drift in *R. padi* populations, the existence of longitudinal clines in the frequencies of the two commonest MLGs of *R. padi* may also involve clonal selection.

The clines in frequency distribution presented above also showed variation in their shape at the beginning and the end of the growing season. Cline shape can vary due to genetic drift and migration (Polechová & Barton, 2011; Rieux *et al.*, 2013) or could result from a change in clonal performance favoring different lineages. Clonal fitness can shift because of environmental variation (Vrijenhoek, 1979) and/or seasonal changes (Weeks & Hoffmann, 1998; Niklasson *et al.*, 2004). First, plant species abundance and/or rotations are major sources of temporal and spatial heterogeneity for crop pests due to differential performance of some lineages according

https://doi.org/10.1017/S0007485315000619 Published online by Cambridge University Press

to the host plant (Vorburger, 2006). However, such host-specialized populations have not been reported so far for *R. padi* (Gilabert *et al*., 2009). Moreover, maize was abundant everywhere along the transect from spring to autumn. It is thus unlikely that temporal and/or spatial variation in frequencies of common MLGs between spring and late summer and along the transect could be due to host selection. Second, variation in cold tolerance has been shown among clones of several aphid species such as *Myzus persicae* (Sulzer, 1776) (Vorburger, 2004) and *Sitobion avenae* (Fabricius, 1775) (Lukasik *et al*., 2011), suggesting that distinct aphid lineages might be selected for according to the season or local climate. This has also been detected in other invertebrates such as in the fly *Dipsa bifurcata* (Niklasson *et al*., 2004), in *Daphnia* species (Jankowski & Straile, 2004; Pinkhaus *et al*., 2007), or in the mite *Penthaleus major* (Dugès, 1834) (Weeks & Hoffmann, 1998). Here, we observed that climate could influence the relative abundance of different *R. padi* MLGs. Although this would require further investigations to replicate the observations over space and time and dissociate the confounding factors, climate could therefore exert strong selective pressures on *R. padi* genotypes. Other important forces such as parasitism pressure may sort out aphid lineages, but their influence could also correlate with longitude and climatic variables in temperate areas as climate can affect the dynamics and the interactions between aphid populations and their pathogens/parasitoids, sometimes through its influence on life-history traits (Le Ralec *et al*., 2010).

A striking result of this study is the opposite changes in the frequencies of the two most common genotypes (*G*1 and *G*2). These were previously characterized as permanently asexual lineages in laboratory experiments, and suggested to be generalist or general-purpose genotypes because of their large tolerance to various climatic conditions and host plant species (Halkett *et al*., 2005; Gilabert *et al*., 2009). The inverted longitudinal clines in their frequency distribution, observed in the present study could however suggest a different ecological range for these two lineages. This opposing pattern of clinal variation in *G*1 and *G*2 may be due to earlier or faster reproductive rate, or opposite adaptations to selective pressures varying with longitude. It could for instance result from difference in thermal preference, with *G*1 having a higher tolerance to grow under low temperature, including a better winter survival, and *G*2 having a higher affinity for mild winter and warm temperatures. Besides difference in thermal preference between genotypes *G*1 and *G*2, both lineages may also vary in their susceptibility to parasitoids or to fungal infections which may be more important with milder temperatures (with genotype *G*2 being more resistant than *G*1). Such a selective advantage of *G*1 when temperatures are low and of *G*2 when temperatures are mild is nevertheless speculative. These changes in genotype abundance could also be amplified by clonal competition. Disentangling the relative contributions of abiotic factors and of between-clones interactions on temporal and spatial variations of abundances of these dominant MLGs would necessitate: (i) testing separately their fitness in different climatic/environmental conditions, and (ii) testing their fitness in the same environment when these MLGs are sympatric.

To conclude, this study reports longitudinal clines in the frequencies of several overrepresented genotypes. The observation of some of these clines at two occasions despite the high level of migration reported in *R. padi* (Delmotte *et al*., 2002; Halkett *et al*., 2005) suggests that drift together with selection could play a role in shaping clonal distribution in that species. From the data presented here, we are currently unable to demonstrate or rule out any of the two hypotheses of clonal distribution in *R. padi* populations of northern France shaped by selection or by genetic drift coupled with migration. To properly disentangle the relative contribution of these forces would require additional temporal and spatial samples combined with simulations and/or experiments in controlled conditions (Campitelli & Stinchcombe, 2013) to control for instance climatic variables such as temperature or humidity. Environmental conditions, including anthropogenic factors (e.g. agricultural practices) have probably favored a few numbers of highly dominant and time persistent clones, each adapted to a given ecological niche. More works are needed on these 'super-clones' to assess their niche breadth and their ecological and evolutionary potential and more generally, to better understand the forces shaping the evolution of asexual or partially asexual species, among which are numerous organisms that cause serious threats to human health, agriculture and environment.

Supplementary Material

The supplementary material for this article can be found at http://www.journals.cambridge.org/BER

Acknowledgements

We would like to thank anonymous reviewers for helpful comments on earlier versions of the manuscript. We thank H. Albert and technical experts from Bayer CropScience – France for providing summer samples. We also thank J. Wasmuth and D. Curran for helpful comments on the manuscript, and G. Mialdea and L. Mieuzet for their help in genotyping. This research was founded by a C.I.F.R.E. grant from the Association Nationale de la Recherche Technique signed with Bayer CropScience.

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