

# Afit: a bioinformatic tool for measuring aphid fitness and invasiveness

A. Nardelli, V. Peona, A. Toschi, M. Mandrioli\* and  
 G.C. Manicardi

Dipartimento di Scienze della Vita, Università di Modena e Reggio Emilia,  
 Via Campi 213/D, 41125 Modena, Italy

## Abstract

A careful measure of fitness represents a crucial target in crop pest management and becomes fundamental considering extremely prolific insects. In the present paper, we describe a standardized rearing protocol and a bioinformatics tool to calculate aphid fitness indices and invasiveness starting from life table data. We tested the protocol and the bioinformatic tool using six *Myzus persicae* (Sulzer) asexual lineages in order to investigate if karyotype rearrangements and ecotype could influence their reproductive performances. The tool showed that different karyotypes do not influence adaptive success and put in evidence a marked invasive potential of the *M. persicae* lineage 64. The presence of a similar fitness rate of 33H and 7GK asexual lineages (both possessing intra-individual karyotype variations) in respect to the asexual lineage 1 (with a standard karyotype) represents an important demonstration of the potentiality of holocentric chromosomes to reduce the effects of chromosome rearrangements.

**Keywords:** aphid, fitness, crowding, forecasting models, invasiveness, karyotype rearrangements

(Accepted 21 October 2016; First published online 22 November 2016)

## Introduction

Fitness estimates the reproductive success and it is equal to the average contribution of the next generation to the gene pool (Benton *et al.*, 2006). Its careful measure represents a crucial target in crop pest insects. For instance, in the case of highly prolific phytophagous insects, such as aphids, these data become fundamental, as they allow to assess the extent of infestations thus enabling proper pest management strategies.

Literature shows a large amount of studies on the adaptive success of many aphid species with an agronomic and/or forestry impact, in connection with both abiotic factors, such as temperature, photoperiod, presence of pollutants, volatile compounds (Kieckhefer & Elliott, 1989; Michels & Behle, 1989; Girma *et al.*, 1990; Hildebrand *et al.*, 1993; Nowierski *et al.*, 1995; Hughes & Bazzaz, 2001; Merrill *et al.*, 2009; Conti *et al.*, 2010), and biotic factors, such as different host plants and

crowding (Judge & Schaefers, 1971; Kidd & Tozer, 1984; Behle & Michels, 1990; Ronquist & Ahman, 1990; Miller *et al.*, 2003; Merrill *et al.*, 2008; Randolph *et al.*, 2008; Ma & Bechinski, 2009; Takaloozadeh, 2010; Taheri *et al.*, 2010; Pucherelli *et al.*, 2011; Mehrparvar *et al.*, 2013; Soffan & Aldawood, 2013; Lu *et al.*, 2016). The effects of ants, predators, parasitoids and symbionts on aphid fitness have also been analyzed (Costello & Altierim, 1995; Chen *et al.*, 2000; Asin & Pons, 2001; Giles *et al.*, 2002; Stadler *et al.*, 2002; Leonardo, 2004; Sakurai *et al.*, 2005; Stadler & Dixon, 2005; Merrill *et al.*, 2009; Ramalho *et al.*, 2015).

The ideal approach to study animal fitness consists of monitoring the biological adaptive traits and reproductive habits of a species within the environment that it naturally inhabits. This method has intrinsic limitations since both the high complexity of ecosystems and the relationships among organisms do hamper a precise evaluation of all the elements influencing the adaptive success of the target. For these reasons, many studies have been based on well-established rearing protocols that allow to control all the external factors, such as weather conditions or presence of predators and parasitoids, enabling to build up representative life tables (i.e., Lu *et al.*, 2016).

Two common fitness indices are the intrinsic rate of population increase  $r_m$ , scaled to time and estimated according to

\*Author for correspondence

Phone: (+39) 059 2055544

Fax: (+39) 059 2055548

E-mail: [mauro.mandrioli@unimo.it](mailto:mauro.mandrioli@unimo.it)

the iterative bisection method from the Euler-Lotka formula (Birch, 1948), and the net reproductive rate  $R_0$  (Stadler *et al.*, 2002) that is scaled per generation and it is independent of the generation time (Stadler *et al.*, 2002).

Aphids have one of the shortest generation times among insects (Taylor, 1981) and therefore the intrinsic rate of increase is the most representative index for measuring their fitness (Taylor, 1981; Tukjapurkar, 1990). However, similarly with other fitness indices,  $r_m$  can vary widely depending on numerous factors, firstly the host plant where aphids are reared (i.e., Lu *et al.*, 2016). Measuring aphid fitness on different host plants in respect to field conditions could therefore lead to under or overestimate their invasiveness. On the other hand, in most natural field settings, the intrinsic rate of increase is problematic to quantify, and so many studies have been carried out till now on aphids considering other fitness components, such as the mean generation time ( $d$ ) and the mean daily fecundity ( $f$ ), or simply comparing the population sizes at different times (Ronquist & Ahman, 1990; Hildebrand *et al.*, 1993; Costello & Altierim, 1995; Hughes & Bazzaz, 2001; Hawley *et al.*, 2003). Several other indices have also been applied in aphids, such as the finite rate of increase  $\lambda$ , calculated as  $\lambda = e^{r_m}$ , the age at which maximum fecundity is achieved,  $X_{\max}$  and the maximum daily fecundity,  $Y_{\max}$  (Stadler *et al.*, 2002), making difficult the comparison of the available data on fitness so that a standardization of the aphid fitness measurement is still missing.

In the present paper we describe a standardized rearing protocol for aphids and a bioinformatics tool (called *Afit*) to calculate aphid fitness indices and invasiveness starting from life table data. In this regard, we developed a new algorithm able to calculate all previously cited indices and specifically: (i) a realistic population size prediction; (ii) a new intrinsic rate of increase of the population ( $r_a$ ); (iii) the crowding effect on population increase ( $r_c$ ).

## Materials and methods

### Pea rearing protocol for *Myzus persicae*

*Myzus persicae* asexual lineages are maintained on pea (*Pisum sativum*) seedlings in a thermostatic room ( $18 \pm 1^\circ\text{C}$ ), as a colony of parthenogenetic females (16 h light and 8 h dark). *M. persicae* asexual lineages 1 and 70 were originally collected on peach *Prunus persica*, lineage 64 was sampled from *Nicotiana tabacum* and 33H was derived from the lineage US1L collected in UK. *M. persicae* asexual lineage 7GK was collected on *Lepidium* sp. in Greece, whereas asexual lineage I-type was collected on *Solanum tuberosum* in Scotland (table 1).

The aphid rearing protocol generally involves a weekly replacement of sprouts. Considering that plants replacement could influence aphid fitness and count, we used plants with a single leaf (the others were removed) and we replaced plants after 12 days. In this way, considering that *M. persicae* usually does not live more than 20 days, we made a single pea plant replacement.

### Life table construction

All the fitness measurements have been carried out on synchronized specimens. To synchronize aphid lineages, we put 10 adult females in jars containing 3 pea shoots. After 24 h we removed them and left new-born nymphs in the jar. In

Table 1. *M. persicae* asexual lineages used for the fitness evaluation.

Asexual lineage	Ecotype	Country	Karyotype	Winged females
1	<i>M. p. Persicae</i>	Italy	$2n = 12$	+
33H	<i>M. p. Persicae</i>	Italy	$2n = 10-15$	-
70	<i>M. p. Persicae</i>	Italy	$2n = 14$	+
7GK	<i>M. p. Persicae</i>	Greece	$2n = 10-17$	-
64	<i>M. p. nicotianae</i>	Italy	tA1-A3	+
I type	<i>M. p. Persicae</i>	Scotland	$2n = 12$	+

this way we get a nymphs pool for which we know the age with 24 h accuracy.

We carried out 5 replicates for each asexual lineage, with each replica consisting of a single synchronized aphid maintained on a plant for each jar. New-born should be removed daily and their number noted. The experiment ended at the death of the  $F_0$  female (table 2). Levene's test was used to assess the equality of variances, precondition to carry out analysis of variance (ANOVA) test.

### Python Afit tool input

The Python bioinformatic tool *Afit* was developed to easily calculate and compare aphid fitness and invasiveness. The tool (freely available at the address <https://moreinsects.net/afit-tool/>) gathers information about the user (name, username, password, name of the institution, email address and notes about the aim of the project), the samples (species, ecotype/subspecies, asexual lineage ID, morph and colour type, host plant, presence of winged morphs, life cycle, insecticide resistance, karyological profile), the sampling locality (State, Province, GPS N-E coordinates) and date and the rearing conditions (host plant, temperature  $^\circ\text{C}$  and photoperiod as day-light length in hours) (fig. 1).

Daily fecundity and crowding data (life tables and population size of each replica) must be recorded on an Excel spreadsheet following the template available as online supplementary material in the downloadable Afitool.zip file.

### Python Afit tool output

The tool generates daily fecundity and population growth curve of the asexual lineage starting from life table data. It calculates clonal fitness indices as a mean of the replicate indices and the SE. Moreover, the tool uses the population size data (obtained starting from any aphid number and measuring at any day) to generate a population growth curve corrected by crowding effect and an advanced intrinsic rate of increase  $r_c$ , corrected by crowding, representative of the crowding sensibility of the asexual lineage (table 3).

### Measuring crowding effect

To assess how crowding affects aphid fecundity, we selected 10 synchronized parthenogenetic females and maintained them in a single jar containing 3 pea plants (3 replicates per asexual lineage).

After 15 days we counted how many aphids were present. The time frame was established on the basis of the average *M. persicae* lifespan in order to obtain a  $F_2$  generation.

Table 2. Upper: daily activities resume needed to set up the life table experiment and collect daily aphid fecundity data. Bottom: daily activities resume needed to set up the experiment and collect aphid crowding data (*i*: seeds imbibition; *s*: synchronization; *x*: jar preparation; *ps*: plantlets substitution; *f*: measure fecundity; *E*: attended end of the experiment).

	Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Life table	Aphids						<i>s</i>	<i>s</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>E</i>
	Seedlings	<i>i</i>						<i>x</i>					<i>ps</i>														
Crowding	Aphids						<i>s</i>	<i>s</i>														<i>E</i>					
	Seedlings	<i>i</i>						<i>x</i>							<i>ps</i>												

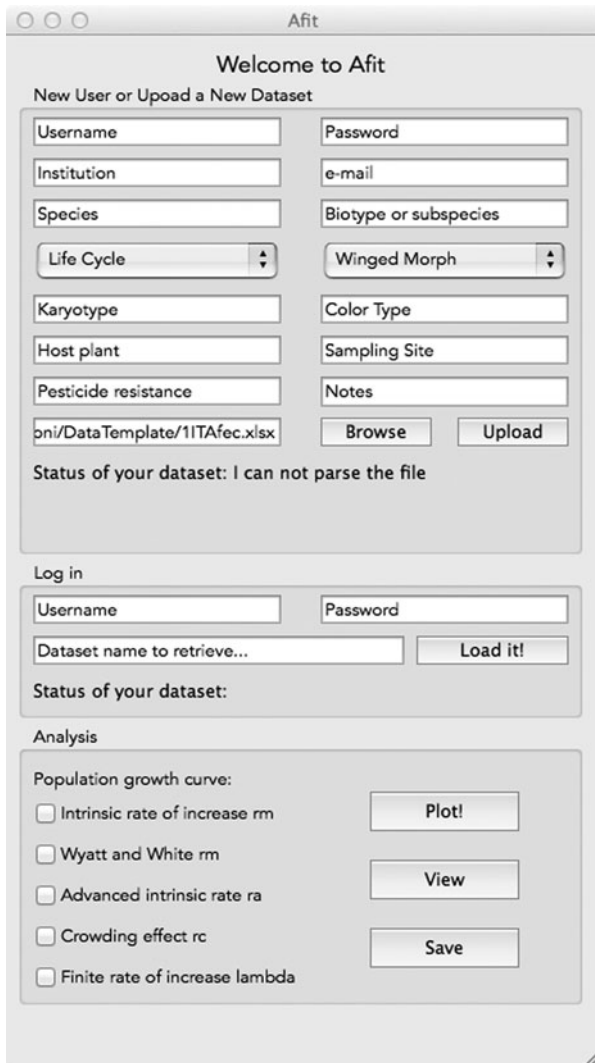


Fig. 1. The Afit tool input window where users can upload their data.

The sprouts were weekly replaced to minimize the plant response to aphid colonization. Experimental crowding data were compared with those predicted by the Python Afit tool, by uploading them and let the software to calculate the percentage decrease of the expected population size at a chosen time. Afit calculated both the population growth curve and the intrinsic rate of increase  $r_c$  corrected by crowding.

Table 3. Fitness indices calculated by the Afit tool and their references and explanation. The software produces a spreadsheet, csv or tsv file for each asexual lineage analyzed where it is possible to find all the biological traits previously uploaded, the daily fecundity and population growth curves and all the fitness indices calculated.

Indices	Description	References
$r_m$	Intrinsic rate of increase	Birch (1948)
$WW-r_m$	Intrinsic rate of increase with correcting constant	Wayne & White (1972)
$r_a$	Advanced intrinsic rate of increase	Nardelli (This paper)
$r_c$	Intrinsic rate of increase corrected by crowding	Nardelli (This paper)
$\lambda$	Finite rate of increase	Wayne & White (1972)
$R_0$	Net reproductive rate	Stadler et al. (2002)
$l$	Longevity	Stadler et al. (2002)
$f$	Mean daily fecundity	Stadler et al. (2002)
$Y_{max}$	Maximum daily fecundity	Stadler et al. (2002)
$X_{max}$	Age at which maximum daily fecundity is achieved	Stadler et al. (2002)

### Results

The pea rearing protocol and the Afit tool have been tested using six *M. persicae* asexual lineages to investigate the effect of different karyotypes rearrangements on their fitness and invasiveness.

A large majority of the analysed *M. persicae* specimens has a lifespan of 15–18 days ( $l$ ) and give birth for the first time ( $d$ ) at day 8 (table 4). Only specimens of the asexual lineage 64 give birth for the first time at day 7 and only I type lives longer than 18 days. The daily mean fecundity ( $f$ ) reaches the highest values with the 64-ita specimens and the lowest with 70.

Clonal daily fecundity curves showed two peaks in the asexual lineages: the first around day 11 and the second one at days 16–17 (fig. 2).

Starting from one aphid, the predicted population growth curves of the six *M. persicae* asexual lineages at day 30 indicated that lineage 64 achieved the highest performance followed by I-type, while 70 had the worst (fig. 3). The asexual lineage 64 reached earlier the highest daily fecundity ( $X_{max}$ ), while the maximum daily fecundity,  $Y_{max}$ , has been achieved by I-type. The asexual lineage 64 reached the highest values for the net reproductive rate value ( $R_0$ ), the intrinsic rate of increase ( $r_m$  and  $r_a$ ) and the finite rate of increase  $\lambda$ , while 70 had the lowest. Intragroup variance is equal according to Levene's test for homogeneity of variance from means and medians ( $P$ -value = 0.2612;  $P$ -value = 0.4114). ANOVA analysis put in evidence statistically significant differences ( $F_{rm} = 2.899$ ;  $P$ -value  $r_m = 0.03475$ ) in fitness costs comparing

Table 4. Life-table and Fitness indices (mean ± SE) produced by *Afit*.

Parameters	1	33H	7GK	64	70	I-type
Intrinsic rate of increase (1/day)	0.292268 ± 0.0138	0.298948 ± 0.0084	0.28853 ± 0.0092	0.33026 ± 0.0100	0.241662 ± 0.0236	0.302028 ± 0.0195
White & Wyatt intrinsic rate of increase (1/day)	0.2864 ± 0.0157	0.2924 ± 0.0102	0.2811 ± 0.0167	0.3288 ± 0.0125	0.218 ± 0.019	0.2778 ± 0.0278
Net reproductive rate (offsprng)	27.6 ± 3.23	27.2 ± 2.70	22.6 ± 3.33	33.4 ± 3.29	14.4 ± 2.51	27.6 ± 4.10
Finite rate of increase (1/day)	1.3401 ± 0.0409	1.3487 ± 0.0246	1.3347 ± 0.0272	1.3917 ± 0.0311	1.2745 ± 0.0545	1.3544 ± 0.0695
Total longevity (day)	17.8 ± 0.9798	17.8 ± 1.1662	15.2 ± 2.3152	17.6 ± 2.5768	16.8 ± 1.6	18.4 ± 1.8547
Mean generation time (day)	8.4 ± 0.4899	8.2 ± 0.4	8 ± 0	7.8 ± 0.4	8.6 ± 0.4899	8.6 ± 1.2
Mean fecundity (offsprng)	2.97797 ± 0.2050	3.18630 ± 0.1589	3.27242 ± 0.1371	3.50119 ± 0.2115	1.71607 ± 0.4730	3.23182 ± 0.7573
Maximum fecundity (offsprng)	6 ± 0.4899	5.2 ± 0.4382	5.4 ± 0.2191	5.75 ± 0.2828	3.8 ± 0.4382	6.4 ± 0.5366
Day of maximum fecundity (day)	11.8 ± 0.5215	12.2 ± 0.4382	10.2 ± 0.3347	9.75 ± 0.1789	10.2 ± 0.5215	10.8 ± 0.6573

asexual lineage 64 (which belongs to *M. persicae nicotianae* ecotype) to the other asexual lineages (which belong to *M. persicae persicae* ecotype).

We also evaluated the crowding effect on female fecundity among aphid lineages (fig. 4) and we noticed a higher population percentage decrease in asexual lineages 64 and I-type (−49.18 and −40.72%, respectively). The lowest value was reached by the asexual lineage 1 (−16.54%).  $r_c$  index followed the  $r_a$  and  $r_m$  trends among asexual lineages (highest within 64 and lower within 70 asexual lineage).

### Discussion

Up till now different parameters have been evaluated in order to adequately explain aphid fitness (e.g., Chen *et al.*, 2000; Stadler *et al.*, 2002; Merrill *et al.*, 2009; Conti *et al.*, 2010; Lu *et al.*, 2016). Mean generation time  $d$ , for instance, strongly affects adaptive success because it confers a lead time during habitat colonization. Longevity is also an important factor because fitness is positively affected when a longer lifespan means a higher net reproductive rate  $R_0$ . On the contrary, if a female lives longer without new-borns, longevity negatively affects fitness, because the female itself could consumes nutritional resources with no regards for her nymphs. The highest values of  $r_m$  and  $r_a$  are achieved by the asexual lineage 64, showing that earliness has greater weight on adaptive success than longevity. Indeed, the asexual lineage I-type lived longer but spawned the same new-borns number compared with other asexual lineages, so that it does not achieve significantly higher fitness indices.

The lowest  $r_m$  and  $r_a$  belong to the asexual lineage 70, while other lineages are on the same, mean equal footing. These features are confirmed by population growth curve trends produced by the *Afit* tool. Although the population percentage decrease are higher in 64 asexual lineage, this lineage has achieved the best fitness performances in crowding conditions.

Fitness indices obtained in this study reached values comparable with those available in literature, however, the diverse rearing and environmental conditions at the basis of the measurement of these indices do not permit a proper comparison between our data and other author's ones.

The use of *Afit* on six different asexual lineages showed similar results also comparing different karyotypes. In particular it has been interesting to evaluate the fitness rate of the two asexual lineages 33H and 7GK possessing recurrent intra-individual chromosomal rearrangements (Manicardi *et al.*, 2015). Chromosome instability is a typical feature of malignant cells, whereas it is a rare condition in physiological situations to such an extent that chromosome number variations are largely utilized for taxonomic and phylogenetic speculations. Aphids represent an exception, since the holocentric nature of their chromosomes could favour karyotype rearrangements. The evidence that fitness rate of 33H and 7GK strains does not differ significantly from asexual lineage 1 (which has a normal karyotype) could result surprising but represent an important demonstration of the potentiality of holocentric chromosomes to reduce the effects of chromosome rearrangements that will be not viable in the presence of monocentric chromosomes.

A further element of interest is related to the presence of the highest fitness rate in the asexual lineage 64 confirming previous field observations concerning the high invasive potential of *M. persicae nicotianae* ecotype (i.e., Kati *et al.*, 2014). Indeed,



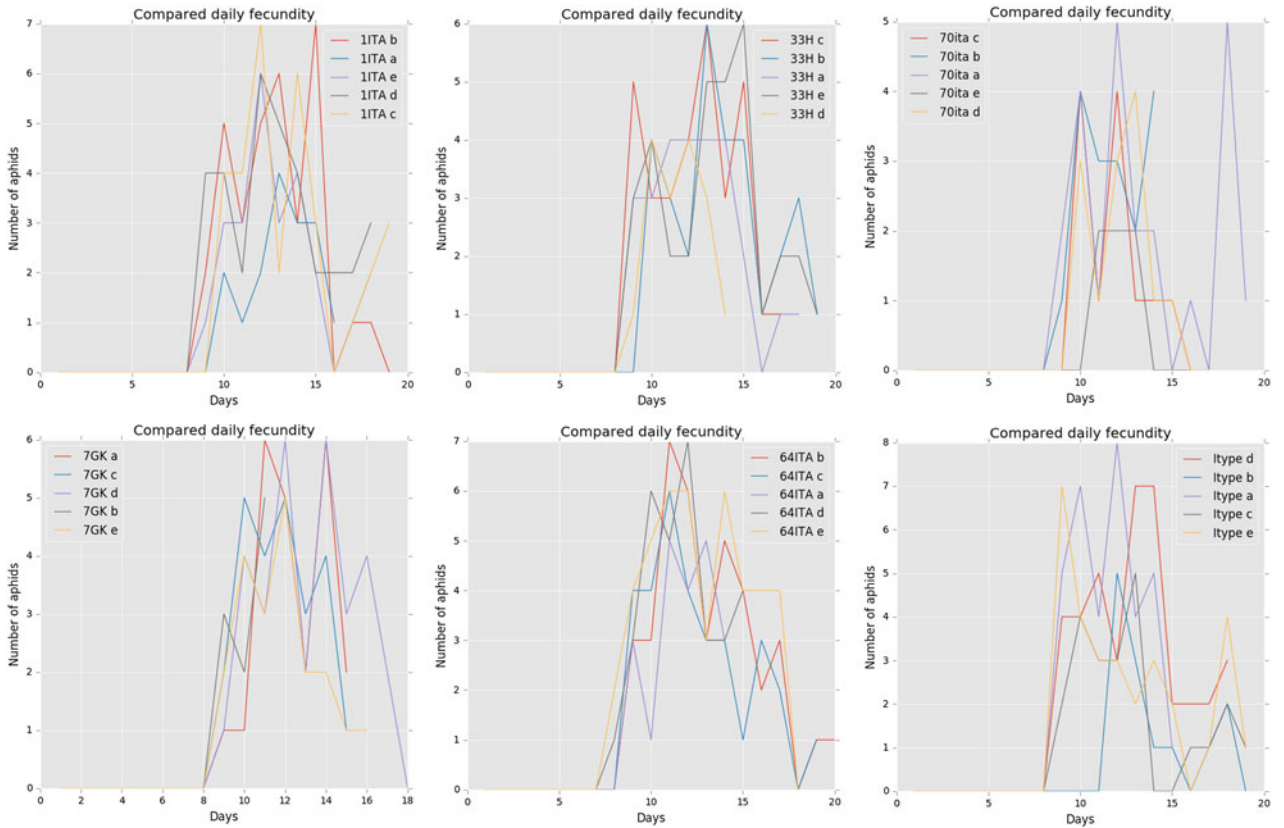


Fig. 2. Fecundity curves of the six *M. persicae* clones produced by *Afit* tool (replicates shown with different colours).

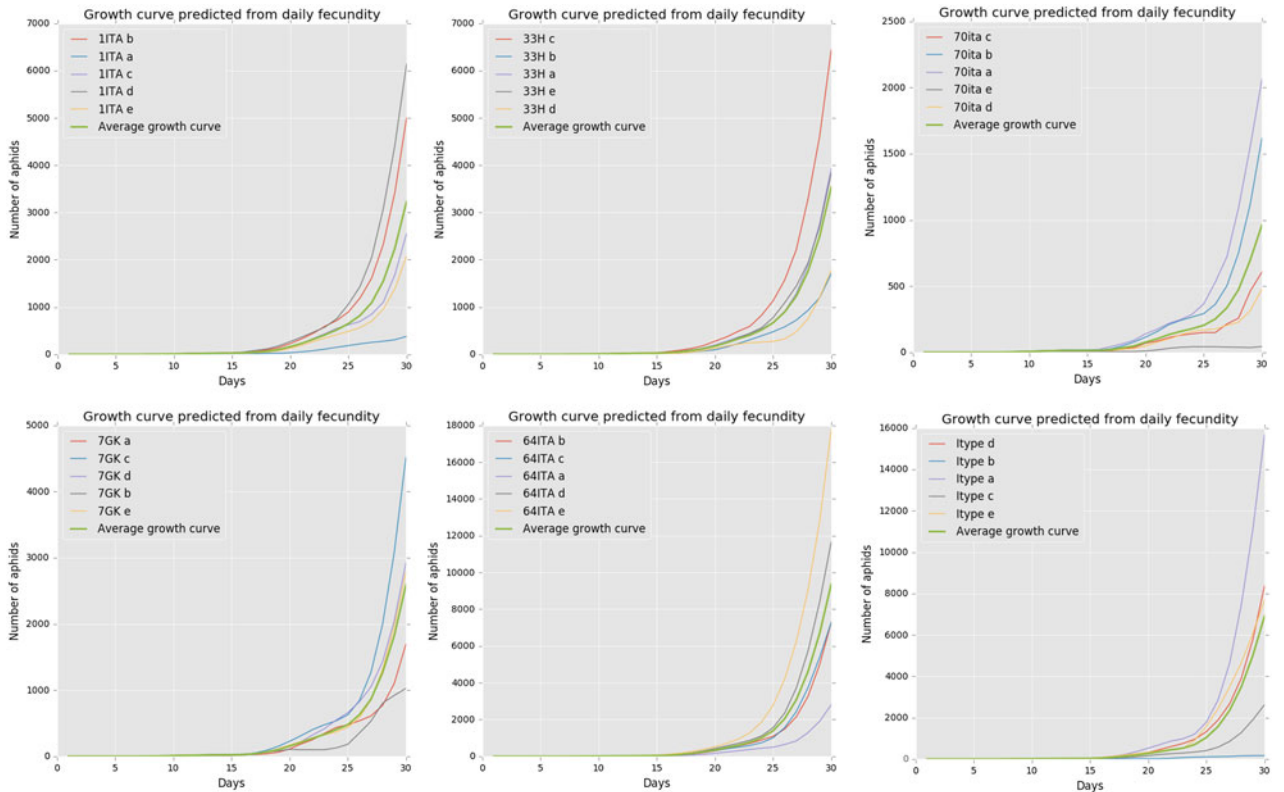


Fig. 3. Population growth curves of the six *M. persicae* clones produced by *Afit* tool.

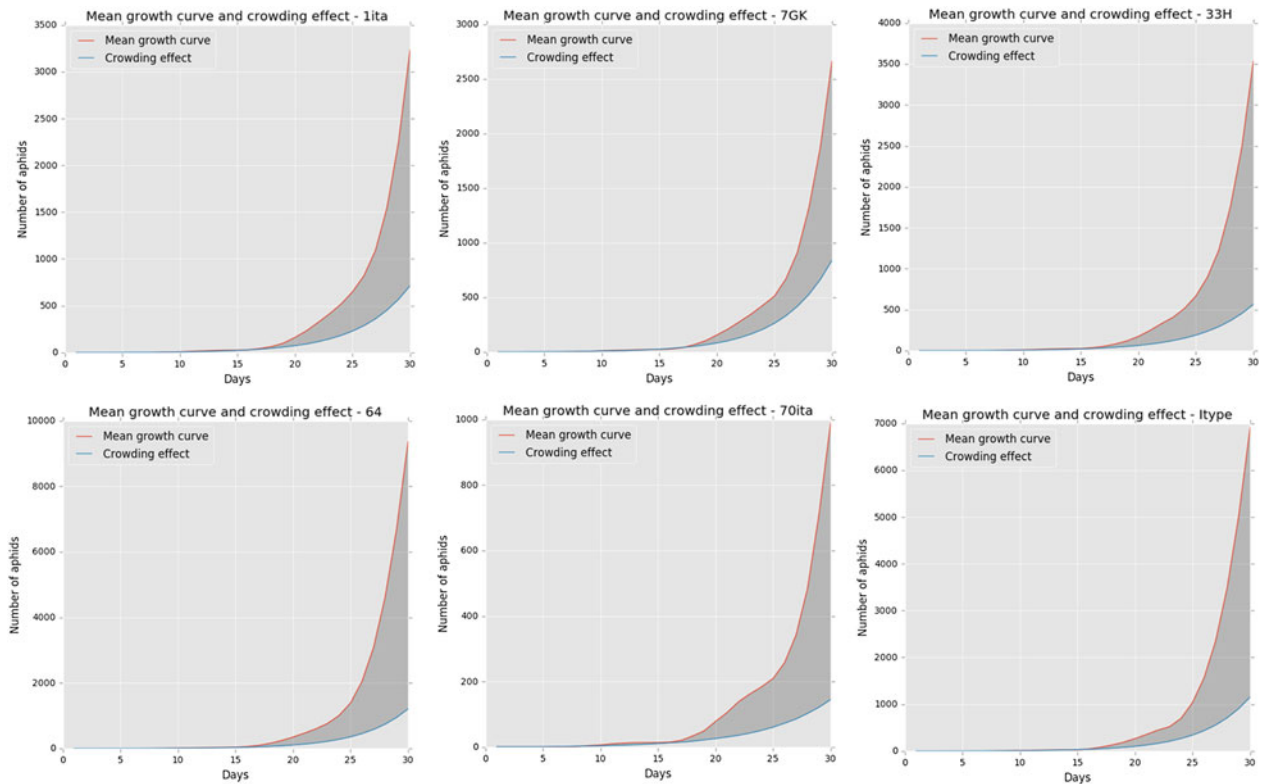


Fig. 4. Population size percentage decrease in a crowded aphid population and the corrected by crowding population growth curve.

Kati *et al.* (2014) reported a rapid spread of this lineage in Greece and provided evidence about rapid evolution in *nicotiana*, which has been suggested as a species ‘in the making’.

The *Afit* tool herein described could be used also for analysis of other parameters such as  $r_m$  established by Wyatt & White (1977). Wayne & White (1972) tried to simplify fitness measuring protocol and proposed a faster method to calculate  $r_m$  assuming that specimens produced in the first 2d time frame, contribute most to the value of  $r_m$  (ca. 95%) (De Loach, 1974). Moreover, they analyzed life table data from four aphid species including *M. persicae* and obtained a correcting constant  $c = 0.738$ . Unfortunately,  $WW-r_m$  cannot be used in the same way as a ‘standard’  $r_m$ . It is pivotal that the patterns of the initial rise and fecundity curves trends among samples should be similar, since the earliest progeny produces the greatest influence on population growth. On the contrary, the value of  $c$  might vary widely. For these reasons White and Wyatt  $r_m$  index tends to be not so representative when used to compare different species or clones with diverse and unpredictable daily fecundity curve trends.

The analysis of the SE within asexual lineages suggests the presence of an intra-clonal variability in the fitness indices. Clonal fitness is therefore not merely a function of survival and fecundity of the daughters alone, but depends on the performance of all descendants up to the time when eggs are fertilized. It means, in other words, that the intrinsic rate of increase only accounts for offspring number, but not for offspring quality. Starting from these assumptions, individual morphs  $r$  values should be carefully considered when an aphid asexual lineage’s life-history is to be explained

(Weisser & Stadler, 1994). This confirms literature data (Lushai & Loxdale, 2002; Erlykova, 2003; Loxdale *et al.*, 2013) suggesting that intra-clonal variability may have effect on different phenotypes, including fitness.

Aphids can produce winged offspring in response to different stimuli, such as crowding (Sutherland 1969a, b; Shaw, 1970), presence of parasitoids (Sloggett & Weisser, 2002), pathogens (Hatano *et al.*, 2012) and natural predators of aphids (Weisser *et al.*, 1999; Kunert & Weisser, 2003). Since winged and un-winged phenotypes are produced by the same genotype, a question for aphid fitness is related to the intrinsic rate of increase  $r$  ( $r_m$  &  $r_a$ ) comparing lineages with different propensity to generate winged offspring. In this view, one of the most interesting applications of our bioinformatics tool will be the measure of the contribution of winged females to population fitness, as well as other parameters, which could influence aphid fitness.

### Acknowledgements

We would like to thank Dr Daniele Baschieri for his support on Phyton scripting and Dr Roberto Simonini for his precious support in the data analysis. We are indebted to colleagues who provided us with *M. persicae* aphid lineages and in particular to Emanuele Mazzoni (Università Cattolica di Piacenza, Italy) for lineages 1, 70, 64 and 33H, John Margaritopoulos (University of Thessaly, Greece) for the lineage 7GK and Brian Fenton (Scotland’s Rural College, Scotland) for the lineage I-type.

## References

- Asin, L. & Pons, X. (2001) Effect of high temperature on the growth and reproduction of corn aphids (Homoptera: Aphididae) and implications for their population dynamics on the North-eastern Iberian Peninsula. *Environmental Entomology* **30**, 1127–1134.
- Behle, R.W. & Michels, G.J. (1990) Russian wheat aphid (Homoptera, Aphididae) development, reproduction, and survival on wheat and rye grown in 4 host-plant media. *Southwestern Entomology* **15**, 109–121.
- Benton, C.T.G., Lundberg, P., Dall, S.R.X., Kendall, B.E. & Gaillard, J.M. (2006) Estimating individual contributions to population growth: evolutionary fitness in ecological time. *Proceedings of the Royal Society B* **273**, 547–555.
- Birch, L.C. (1948) The intrinsic rate of natural increase of an insect population. *Animal Ecology* **17**, 15–26.
- Chen, D.Q., Montllor, C.B. & Purcell, A.H. (2000) Fitness effects of two facultative endosymbiotic bacteria on the pea aphid, *Acyrtosiphon pisum*, and the blue alfalfa aphid, *A. kondoi*. *Entomologia Experimentalis et Applicata* **95**, 315–323.
- Conti, B., Bueno, V., Sampaio, M. & Sidney, L. (2010) Reproduction and fertility life table of three aphid species (Macrosiphini) at different temperatures. *Revista Brasileira de Entomologia* **54**, 654–660.
- Costello, M.J. & Altierim, M.A. (1995) Abundance, growth rate and parasitism of *Brevicoryne brassicae* and *Myzus persicae* (Homoptera: Aphididae) on broccoli grown in living mulches. *Agriculture, Ecosystems and Environment* **52**, 187–196.
- De Loach, C.J. (1974) Rate of increase of populations of cabbage, green peach, and turnip aphids at constant temperatures. *Annals of the Entomological Society of America* **67**, 332–340.
- Erlykova, N. (2003) Inter- and intra-clonal variability in the photoperiodic response and fecundity in the pea aphid *Acyrtosiphon pisum* (Homoptera: Aphididae). *European Journal of Entomology* **100**, 31–37.
- Giles, K.L., Madden, R.D., Stockland, R., Payton, M.E. & Dillwith, J.W. (2002) Host plants affect predator fitness via the nutritional value of herbivore prey: investigation of a plant-aphid-ladybeetle system. *BioControl* **47**, 1–21.
- Girma, M., Wilde, G. & Reese, J.C. (1990) Influence of temperature and plant-growth stage on development, reproduction, life-span, and intrinsic rate of increase of the Russian wheat aphid (Homoptera, Aphididae). *Environmental Entomology* **19**, 1438–1442.
- Hatano, E., Baverstock, J., Kunert, G., Pell, J.K. & Weisser, W.W. (2012) Entomopathogenic fungi stimulate transgenerational wing induction in pea aphid *Acyrtosiphon pisum* (Homoptera: Aphididae). *Ecological Entomology* **37**, 75–82.
- Hawley, C.J., Peairs, F.B. & Randolph, T.L. (2003) Categories of resistance at different growth stages in halt, a winter wheat resistant to the Russian wheat aphid (Homoptera: Aphididae). *Journal of Economical Entomology* **96**, 214–219.
- Hildebrand, D.F., Brown, G.C., Jackson, D.M. & Hamilton-Kemp, T.R. (1993) Effects of some leaf emitted volatile compounds on aphid population increase. *Journal of Chemical Ecology* **19**, 1875–1887.
- Hughes, L. & Bazzaz, F.A. (2001) Effects of elevated CO<sub>2</sub> on five plant-aphid interactions. *Entomologia Experimentalis et Applicata* **99**, 87–96.
- Judge, F.D. & Schaeffers, G.A. (1971) Effects of crowding on alary polymorphism in the aphid *Chaetosiphon fragaefolii*. *Journal of Insect Physiology* **17**, 143–148.
- Kati, A.N., Mandrioli, M., Skouras, P.J., Malloch, G.L., Voudouris, C.Ch., Venturrelli, M., Manicardi, G.C., Tsitsipis, J.A., Fenton, B. & Margaritopoulos, J.T. (2014) Recent changes in the distribution of carboxylesterase genes and associated chromosomal rearrangements in Greek populations of the tobacco aphid *Myzus persicae nicotianae*. *Biological Journal of the Linnean Society* **113**, 455–470.
- Kidd, N.A.C. & Tozer, D.J. (1984) Host plant and crowding effects in the induction of alatae in the large pine aphid, *Cinara pinea*. *Entomologia Experimentalis et Applicata* **35**, 37–42.
- Kieckhefer, R.W. & Elliott, N.C. (1989) Effect of fluctuating temperatures on development of immature Russian wheat aphid (Homoptera, Aphididae) and demographic statistics. *Journal of Economical Entomology* **82**, 119–122.
- Kunert, G. & Weisser, W.W. (2003) The interplay between density- and trait-mediate effects in predator-prey interactions: a case study in aphid wing polymorphism. *Oecologia* **135**, 304–312.
- Leonardo, T.E. (2004) Removal of a specialization-associated symbiont does not affect aphid fitness. *Ecology Letters* **7**, 461–468.
- Loxdale, H.D., Vorwek, S. & Forneck, A. (2013) The unstable 'clone': evidence from monitoring AFLP-based mutations for short-term clonal genetic variation in two asexual lineages of the grain aphid, *Sitobion avenae* (F.). *Bulletin of Entomological Research* **103**, 11–18.
- Lu, H., Yang, P., Xu, Y., Luo, L., Zhu, J., Cui, N., Kang, L. & Cui, F. (2016) Performances of survival, feeding behavior, and gene expression in aphids reveal their different fitness to host alteration. *Scientific Reports* **6**, 19344.
- Lushai, G. & Loxdale, H.D. (2002) The biological improbability of a clone. *Genetical Research* **79**, 1–9.
- Ma, Z. & Bechinski, E. (2009) Life tables and demographic statistics of Russian wheat aphid (Homoptera: Aphididae) reared at different temperatures and on different host plant growth stages. *European Journal of Entomology* **106**, 205–210.
- Manicardi, G.C., Nardelli, A. & Mandrioli, M. (2015) Fast chromosomal evolution and karyotype instability: recurrent chromosomal rearrangements in the peach potato aphid *Myzus persicae* (Homoptera: Aphididae). *Biological Journal of the Linnean Society* **116**, 519–529.
- Mehrpour, M., Zytynska, S.E. & Weisser, W.W. (2013) Multiple cues for winged morph production in an aphid meta-community. *PLoS ONE* **8**, e58323.
- Merrill, S., Peairs, F.B., Miller, H.R., Randolph, T.L., Rudolph, J. B. & Talmichm, E.E. (2008) Reproduction and development of Russian wheat aphid Biotype 2 on crested wheatgrass, intermediate wheatgrass, and susceptible and resistant wheat. *Journal of Economical Entomology* **101**, 541–545.
- Merrill, S., Holtzer, T. & Peairs, F.B. (2009) *Diuraphis noxia* reproduction and development with a comparison of intrinsic rates of increase to other important small grain aphids: a meta-analysis. *Environmental Entomology* **38**, 1061–1068.
- Michels, G.J. & Behle, R.W. (1989) Influence of temperature on reproduction, development, and intrinsic rate of increase of Russian wheat aphid, Green-bug, and Bird cherry-oat aphid (Homoptera, Aphididae). *Journal of Economical Entomology* **82**, 439–444.
- Miller, H.R., Randolph, T.L. & Peairs, F.B. (2003) Categories of resistance at four growth stages in three wheats resistant to the Russian wheat aphid (Homoptera: Aphididae). *Journal of Economical Entomology* **96**, 673–679.
- Nowierski, R.M., Zeng, Z. & Scharen, A.L. (1995) Age specific life table modelling of the Russian wheat aphid (Homoptera,

- Aphididae) on barley grown in benzimidazole agar. *Environmental Entomology* **24**, 1284–1290.
- Pucherelli, S., Peairs, F., Merrill, S. & Randolph, T.** (2011) Russian wheat aphid (Hemiptera: Aphididae) reproduction and development on five non cultivated grass hosts. *Arthropod-Plant Interactions* **6**, 67–73.
- Ramvalho, F., Malaquias, J., Lira, A., Oliveira, F., Zanuncio, J. & Fernandes, F.** (2015) Temperature-dependent fecundity and life table of the fennel aphid *Hyadaphis foeniculi* (Passerini) (Hemiptera: Aphididae). *PLoS ONE* **10**, e0122490.
- Randolph, T.L., Merrill, S. & Peairs, F.B.** (2008) Reproductive rates of Russian wheat aphid (Hemiptera: Aphididae) biotypes 1 and 2 on a susceptible and a resistant wheat at three temperature regimes. *Journal of Economic Entomology* **101**, 955–958.
- Ronquist, F. & Ahman, I.** (1990) Reproductive rate of the Indian mustard aphid (*Lipaphis erysimi pseudobrassicae*) on different *Brassica* oilseeds: comparisons with Swedish strains of mustard (*Lipaphis erysimi erysimi*) and cabbage aphid (*Brevicoryne brassicae*). *Annals of Applied Biology* **116**, 425–430.
- Sakurai, M., Koga, R., Tsuchida, T., Meng, X. & Fukatsu, T.** (2005) *Rickettsia* symbiont in the pea aphid *Acyrtosiphon pisum*: novel cellular tropism, effect on host fitness, and interaction with the essential symbiont *Buchnera*. *Applied and Environmental Microbiology* **71**, 4069–4075.
- Shaw, M.J.P.** (1970) Effects of population density on alienicolae of *Aphis fabae* Scop. I. Effect of crowding on production of alatae in laboratory. *Annals of Applied Biology* **65**, 191–196.
- Sloggett, J.J. & Weisser, W.W.** (2002) Parasitoids induce production of the dispersal morph of the pea aphid *Acyrtosiphon pisum*. *Oikos* **98**, 323–333.
- Soffan, A. & Aldawood, A.** (2014) Biology and demographic growth parameters of cowpea aphid (*Aphis craccivora*) on faba bean (*Vicia faba*) cultivars. *Journal of Insect Science* **14**, 120–121.
- Stadler, B. & Dixon, A.F.G.** (2005) Ecology and evolution of aphid-ant interactions. *Annual Review of Ecology, Evolution and Systematics* **36**, 345–372.
- Stadler, B., Dixon, A.F.G. & Kindlmann, P.** (2002) Relative fitness of aphids: effects of plant quality and ants. *Ecology Letters* **5**, 216–222.
- Sutherland, O.R.W.** (1969a) The role of crowding in the production of winged forms by two strains of the pea aphid, *Acyrtosiphon pisum*. *Journal of Insect Physiology* **15**, 1385–1410.
- Sutherland, O.R.W.** (1969b) The role of the host plant in the production of winged forms by two strains of the pea aphid, *Acyrtosiphon pisum*. *Journal of Insect Physiology* **15**, 2179–2201.
- Taheri, S., Razmjou, J. & Rastegari, N.** (2010) Fecundity and development rate of the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Homoptera: Aphididae) on six wheat cultivars. *Plant Protection Science* **46**, 72–78.
- Takaloozadeh, H.M.** (2010) Effects of host plants and various temperatures on population growth parameters of *Aphis gossypii* Glover (Homoptera: Aphididae). *Middle-East Journal of Scientific Research* **6**, 25–30.
- Taylor, F.** (1981) Ecology and evolution of physiological time in insects. *American Naturalist* **117**, 1–23.
- Tukjapurkar, S.** (1990) Delayed reproduction and fitness in variable environments. *Proceedings of the National Academy of Science USA* **87**, 1139–1143.
- Weisser, W.W. & Stadler, B.** (1994) Phenotypic plasticity and fitness in aphids. *European Journal of Entomology* **91**, 71–78.
- Weisser, W.W., Braendle, C. & Minoretti, N.** (1999) Predator-induced morphological shift in the pea aphid. *Proceedings of the Royal Society B: Biological Sciences* **266**, 1175–1181.
- Wyatt, I. & White, P.** (1977). Simple estimation of intrinsic increase rates for aphids and tetranychid mites. *Journal of Applied Ecology* **14**, 757–766.