

5-26-2009

Similar Patterns of Linkage Disequilibrium and Nucleotide Diversity in Native and Introduced Populations of the Pea Aphid, *Acyrtosiphon pisum*

Jennifer A. Brisson

University of Nebraska - Lincoln, jbrisson2@unl.edu

Sergey V. Nuzhdin

University of Southern California, Los Angeles, snuzhdin@usc.edu

David L. Stern

Princeton University, dstern@princeton.edu

Follow this and additional works at: <http://digitalcommons.unl.edu/bioscifacpub>



Part of the [Life Sciences Commons](#)

Brisson, Jennifer A.; Nuzhdin, Sergey V.; and Stern, David L., "Similar Patterns of Linkage Disequilibrium and Nucleotide Diversity in Native and Introduced Populations of the Pea Aphid, *Acyrtosiphon pisum*" (2009). *Faculty Publications in the Biological Sciences*. 38. <http://digitalcommons.unl.edu/bioscifacpub/38>

This Article is brought to you for free and open access by the Papers in the Biological Sciences at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Faculty Publications in the Biological Sciences by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Research article

Open Access

Similar patterns of linkage disequilibrium and nucleotide diversity in native and introduced populations of the pea aphid, *Acyrtosiphon pisum*

Jennifer A Brisson*^{1,4}, Sergey V Nuzhdin² and David L Stern³

Address: ¹Section of Ecology and Evolution, University of California at Davis, Davis, CA, USA, ²Computational and Molecular Biology, University of Southern California, Los Angeles, CA, USA, ³Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ, USA and ⁴Current address: Computational and Molecular Biology, University of Southern California, Los Angeles, CA, USA

Email: Jennifer A Brisson* - jennifer.brisson@gmail.com; Sergey V Nuzhdin - snuzhdin@usc.edu; David L Stern - dstern@princeton.edu

* Corresponding author

Published: 26 May 2009

Received: 27 June 2008

BMC Genetics 2009, 10:22 doi:10.1186/1471-2156-10-22

Accepted: 26 May 2009

This article is available from: <http://www.biomedcentral.com/1471-2156/10/22>

© 2009 Brisson et al; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Background: The pea aphid, *Acyrtosiphon pisum*, is an emerging genomic model system for studies of polyphenisms, bacterial symbioses, host-plant specialization, and the vectoring of plant viruses. Here we provide estimates of nucleotide diversity and linkage disequilibrium (LD) in native (European) and introduced (United States) populations of the pea aphid. Because introductions can cause population bottlenecks, we hypothesized that U.S. populations harbor lower levels of nucleotide diversity and higher levels of LD than native populations.

Results: We sampled four non-coding loci from 24 unique aphid clones from the U. S. (12 from New York and 12 from California) and 24 clones from Europe (12 alfalfa and 12 clover specialists). For each locus, we sequenced approximately 1 kb from two amplicons spaced ~10 kb apart to estimate both short range and longer range LD. We sequenced over 250 kb in total. Nucleotide diversity averaged 0.6% across all loci and all populations. LD decayed slowly within ~1 kb but reached much lower levels over ~10 kb. Contrary to our expectations, neither LD nor nucleotide diversity were significantly different between native and introduced populations.

Conclusion: Both introduced and native populations of pea aphids exhibit low levels of nucleotide diversity and moderate levels of LD. The introduction of pea aphids to North America has not led to a detectable reduction of nucleotide diversity or increase in LD relative to native populations.

Background

One important goal of evolutionary biology is to identify the genetic causes of phenotypic variation in wild populations. In theory, in large, randomly mating populations where all loci are in linkage equilibrium, genotype can be connected to phenotype by assaying every DNA polymorphism and associating those polymorphisms with phenotypic variation [1]. In practice, loci are often in linkage

disequilibrium (LD) and not all polymorphisms can be surveyed due to cost. LD can be exploited, however, to identify genomic regions, rather than individual nucleotides, that are associated with phenotypic variation [e.g., [2]]. The scale at which polymorphisms must be sampled depends on the level and decay of LD across the genome. Higher levels of LD allow shallower sampling of polymorphisms, but provide reduced resolution for locating causal

variants. Lower levels of LD force denser sampling of polymorphisms, but provide greater precision for linking genotype to phenotype.

Systematic studies of LD have been performed for few species and mainly for plants. Among plants, LD varies widely, with higher levels observed in predominately selfing organisms such as *Arabidopsis thaliana* [3,4] and soybeans [5]. Selfing reduces effective heterozygosity and therefore the ability of recombination to reduce LD. Among outcrossers such as sunflowers [6] and maize [7,8], LD decays more rapidly, reaching background levels within one kilobase. In humans LD persists from tens to hundreds of kilobases [2,9,10]. In a flycatcher (*Ficedula albicollis*), significant LD extends over 400 to 500 kb [11]. In *Caenorhabditis elegans* LD generally decays to insignificant levels within a kilobase [12,13] and in *Drosophila melanogaster* within even shorter distances [14].

Here we characterize levels of LD and nucleotide diversity in the pea aphid (*Acyrtosiphon pisum*). Pea aphids, like most aphids, are cyclical parthenogens [15]. During the spring and summer months, females are parthenogenetic, giving live birth to typically genetically identical daughters via a modified meiosis lacking genetic recombination [16] (but see [17] for a recent review of the possibility of genetic variation within an aphid clone). A lineage of asexual females is typically referred to as a clone. Pea aphid males and sexual females are produced asexually in the fall in response to shortened day-length and lower temperatures [18,19]. Eggs produced by the sexual generation diapause through the cold winter months when adults perish.

The pea aphid genome has been sequenced at 6× coverage by the Human Genome Sequencing Center at Baylor College of Medicine and an assembly has been released <http://www.hgsc.bcm.tmc.edu/projects/aphid>. Notable aspects of the pea aphid's biology include switching seasonally between asexual and sexual reproduction [15], harboring obligate and facultative bacterial endosymbionts [20,21], producing winged and unwinged morphs in response to environmental cues [22], displaying strong preferences for different host plants [23], transmitting plant viral diseases [24], and producing overwintering, diapausing eggs [25]. Many of these traits exhibit variable phenotypes that can be explored using a mapping approach, which would be informed by estimates of levels of nucleotide diversity and LD in this species.

Pea aphids infest field crops of the pea family such as alfalfa (genus *Medicago*) and clover (genus *Trifolium*). Although Eurasian in origin, the pea aphid has been introduced to North America, presumably via agriculture, probably many times over the last 200 years [26]. Severe

bottleneck events, such as those associated with the introduction of only a few individuals, are expected to dramatically decrease levels of nucleotide diversity and increase levels of LD [27,28]. Less severe bottlenecks decrease the number of low-frequency single-nucleotide polymorphisms (SNPs) in the introduced population [29]. We therefore hypothesized that introduced populations harbor lower levels of nucleotide diversity and higher levels of LD than native populations. If true, the two populations would have different utility for the coarse-mapping (introduced) and fine-mapping (native) of phenotypic variation.

Aphid clones display increased survivorship and fecundity on their preferred host plants. In New York, pea aphids specialize on alfalfa (*Medicago sativa*) or red clover (*Trifolium pratense*) [23,30] and this specialization has a genetic basis [31]. Specialization on alfalfa, clover and other species of the pea family has also been observed in France [32], Sweden [33], and the United Kingdom [34]. In California, pea aphids specialize on white clover (*Trifolium repens*), but no alfalfa specialists have been reported. Clones found on alfalfa are more generalized in that they are able to feed on either clover or alfalfa [35]. We sequenced four loci from pea aphid clones from both alfalfa and clover in Europe and alfalfa in the U. S. to test whether host plant specialization or population origin was associated with population differentiation at the sampled loci and with different patterns of LD or nucleotide diversity.

Methods

Sampling

Twelve pea aphid clones were collected from alfalfa fields in Yolo and Galt counties of central California by T. Leonardo and S. Nuzhdin [35]. Twelve clones were collected from alfalfa fields in Western and Central France (Domagné and Lusignan) by J. C. Simon. Two clones were collected from clover fields in Germany (coordinates 50°54' W 11°33'N and 50°59' W 11°31'N) and ten from clover fields in France (coordinates 48°06' W 1°47'N, as well as Domagné and Lusignan) by J. C. Simon. Finally, 12 New York clones were collected from alfalfa fields in Tompkins county by M. Caillaud and Otsego county by J. Brisson. From each clone, we extracted DNA from two to six females using the Qiagen (Valencia, CA) DNAeasy kit.

Loci Studied

We assembled four contiguous segments of 10 to 20 kb of genomic DNA using sequences from the NCBI trace archive. Two of these loci, CAA and 380, are located on the X chromosome while the other two, 870 and 41, are on autosomes. Pea aphids have three autosomes and one large X chromosome that appears to contain about a third of the nuclear genome content [16]. Small sequence sub-

sets of these 10 to 20 kb segments were previously identified for the CAA, 380 and 870 loci [31,36], while 41 was developed in this study.

We wanted to assay LD within short distances (less than 1 kb) and longer distances (over about 10 kb), so we designed two sets of PCR primers for each of the four loci. The resulting amplicons from each set were spaced by about 10 kb. Because we had two amplicons per locus and four loci, in total we utilized eight amplicons (from each of the 48 clones). Specifically, our amplicons (sized between 500 and 1000 bp) were separated by 9249 bp for locus 380, by 11371 bp for locus 870, by 9163 bp for locus 41, and by 10684 bp for locus CAA. After release of the first assembly of the pea aphid genome, Acyr 1.0, we confirmed these distances, with locus 380 on contig 7746 in scaffold 5539, 870 on contig 14350 in scaffold 14350, 41 on contig 6316 in scaffold 12009 and CAA on contig 18522 in scaffold 15751. We also confirmed that all four of the loci are in intergenic regions. Primers are listed in Table 1. 50 μ l PCR reactions included 25 μ l of 2 \times PCR Master Mix (Fermentas, Glen Burnie, MD), 5 μ l of each 10 μ M primer, 2 μ l of genomic DNA, and 13 μ l H₂O. All PCR reactions were done with negative controls under the following conditions: an initial 95°C anneal, 30 cycles of 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 1 minute, with a final 10 minute extension at 72°C. PCR products were purified using the Invitrogen (Carlsbad,

CA) Purelink PCR purification kit and sequenced directly from both ends as well as with the internal primers listed in Table 1.

Sequence Analyses

Sequences were assembled using CodonCode Aligner (CodonCode Corporation, Dedham, MA). Because these were natural pea aphid isolates, all loci had heterozygous positions and some had heterozygous insertions or deletions (indels). The software identified heterozygous positions that were confirmed by visual inspection of the sequence trace file. In some cases we did not sequence across the entire amplicon due to the presence of heterozygous indels. These areas were treated as unknown data. There were also instances where we were unable to amplify a locus in a given genotype. Figure 1 illustrates the gaps in our data for each of the four loci. All indels were excluded from analyses of nucleotide diversity. Sequences have been deposited in GenBank under accession numbers [FJ825706–FJ825751](#) and [FJ858381–FJ858708](#).

Analyses of LD were performed using the Genetics package [37] in the R statistical computing environment [38]. Our data are genotypic, not haplotypic data. Thus, for two loci, A and B, AB/ab is indistinguishable from Ab/aB. The frequency of AB, p_{AB} , in this package is estimated via maximum likelihood, and then used to compute the difference in expected versus observed allele pairs, D , where D

Table 1: Primers used for PCR and sequencing.

Locus	Forward primer	Reverse primer
CAA-1	TAAACGTTTCGGTTTCTGTTCC	AGGGATATCCAGGATGGTTTG
CAA-2	TACATTGCGCACTATAAATTAGGG	ATTAAGTCACCAACCTTTTCCAAC
380-1	ACGGTATCGAATCCCCTTAAAAAC	AGATACCGCAGAACTAGTGGAAATG
380-2	TAAAGCCGCATCCTATAAACTCTC	TAATACGGTCGTCTGTCACAACCTC
380-int1	TTCGAATGTAATTTATTACCAGG	
380-int2	CCTCTAAATACGGCCCTGATGAACC	
380-int3	AAACTGTTTAACTCACAAAG	
870-1	CTATGTGGATGTTGATACCGAAAG	TTCCGTATGCTATATTTCCGTCTG
870-2	AACTTCTAAATACCGGACAATCTCG	CAAACCTGTCCTCTGAGAAATGAGAGG
870-int	TGAAAGCCCAGACGTCGATGCTAGC	
41-1	CTTCTGCCAGTCACAAACAATC	CGATGAAGATCTTTCAGTGCAC
41-2	AACTAAGCCGTCTGCGTTACTC	CATTTACACGGTACCGACATTG

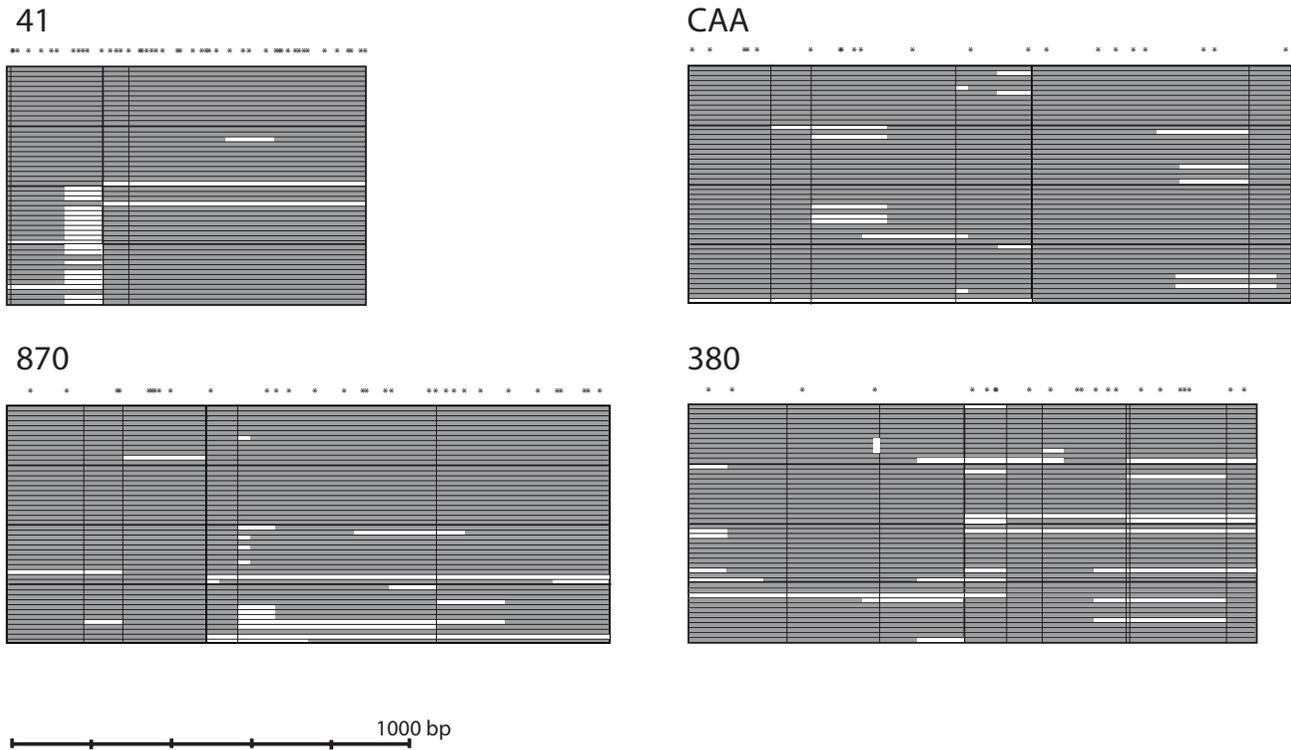


Figure 1
Summary of the sequence data used in this study for the two autosomal (left, 41 and 870) and two X-linked (right, CAA and 380) loci. Each locus consisted of two sequenced segments of DNA separated by several kilobases (9.2 kb for locus 41, 11.4 kb for locus 870, 10.7 kb for locus CAA, and 9.2 kb for locus 380). A heavy black vertical line separates the two sequenced segments at each locus. Each locus is additionally broken up into the four different populations, indicated by four horizontal cells separated by heavy black lines. The California population is on top, then the New York, Europe alfalfa, and lastly Europe clover. There are 12 individuals per cell, each separated by a fine black horizontal line. Grey shading indicates that the sequence data were present; white indicates missing data (primarily due to heterozygous indels, see text). Above the boxes are asterisks that specify the approximate sequence position of SNPs. Fine black vertical lines indicate the direct observation of an indel at that position. A scale bar is given at the bottom of the diagram.

= $p_{AB} - p_A p_B$ and the correlation coefficient between the pairs, r , where $r = -D / (\sqrt{(p_A p_a p_B p_b)})$. We thus report LD values between pairs of sites using the squared allele frequency correlation measure, r^2 . Rare sites (where only one individual differed from the others) were excluded prior to computations. Significance of LD between SNPs was assessed using Fisher's exact test, also implemented using the Genetics package. To estimate the decay of LD over distance, we used nonlinear regression of LD between polymorphic sites versus physical distance [7]. The expected value of r^2 using a drift-recombination model is $E(r^2) = 1 / (1 + C)$, where C equals the population recombination parameter ($C = 4N_e r$). Adding mutation to the model, the expectation becomes:

$$E(r^2) = ((10 + C) / (2+C)(11+C)) / (1+(3+C)(12+12C+C^2) / (n(2+C)(11+C))), [39]$$

where n is the number of sequences sampled. We fit this model after replacing C by $C \cdot \text{dis-}$

tance in base pairs between sites using PROC NLIN in SAS [40].

We calculated π , θ , and Tajima's D using R. π measures nucleotide diversity as the average number of nucleotide differences per site between all pairwise comparisons of sequences and θ is the level of heterozygosity determined by the number of variable positions [41,42]. Tajima's D is a measure of whether the frequency distribution of segregating sites at a locus is consistent with neutral expectations [43]. Levels of population differentiation (F_{st}) for each of the four loci were estimated using Genepop [44], which uses a weighted analysis of variance [45]. We also used Genepop to determine whether any of the loci were not in Hardy-Weinberg equilibrium, testing the null hypothesis of random union of gametes using the exact Hardy-Weinberg test [46].

Results

Sequence Diversity

To measure levels of sequence diversity and LD decay over short distances (less than 1 kb) as well as over longer distances (about 10 kb), we sequenced two amplicons spaced by about 10 kb at four different genomic regions for 12 clones from New York, 12 clones from California, and 24 clones from Europe (22 clones from France and two clones from Germany). The European clones consisted of 12 alfalfa and 12 clover specialists. Two of the genomic regions were from autosomes (41 and 870) and two were from the X chromosome (380 and CAA). A total of approximately 5452 bp was sequenced for each individual, with over 250 kb sequenced in total. None of the regions included coding sequence and all were in intergenic regions as determined by querying the "nr" (non-redundant) database with a blastX (translated versus protein database) search of their associated genomic contigs. We excluded all indel information from our analyses

because we used diploid individuals and could not precisely assay the number or size of indels in some of our individuals. Heterozygous indels in some cases led to missing sequence data (see Figure 1).

Table 2 reports estimates of nucleotide diversity (π) and Watterson's θ . Nucleotide diversity was significantly lower on the X chromosome than the autosomes (1-sided $P < 0.001$, unpaired t-test comparing the eight autosomal π values to the eight X π values; if we increase the sample size contributing to nucleotide diversity estimates by calculating a π value for the North American samples and a π value for the European samples for each locus and then compare the four autosomal π values to the four X π values, 1-sided $P < 0.01$). Further, measures of nucleotide diversity were not significantly different between North American and European populations (NY and CA π values averaged versus Europe, alfalfa and clover averaged: 1-sided $P = 0.12$, paired t-test; again, if we calculate a π value

Table 2: Measures of nucleotide diversity, Tajima's D, and LD.

Locus	Bp	Population	S ^A	Pi	Theta	D	Mean r ² short distances	Mean r ² longer distances
41	928	California	26(0)	0.0108	0.0061	2.88**	0.31	0.198
		New York	33(10)	0.0111	0.0087	1.09	0.215	0.187
		Europe, alfalfa	33(17)	0.0091	0.0083	0.37	0.275	0.141
		Europe, clover	38(16)	0.0116	0.0093	0.92	0.193	0.217
870	1487	California	30(13)	0.0061	0.0055	0.45	0.458	0.300
		New York	28(11)	0.0053	0.0049	0.31	0.353	0.044
		Europe, alfalfa	31(2)	0.0093	0.0056	2.54**	0.408	0.167
		Europe, clover	27(4)	0.0072	0.0046	2.19*	0.716	0.159
CAA	1608	California	6(5)	0.0008	0.0010	-0.65	NA	NA
		New York	14(3)	0.0028	0.0020	1.34	0.221	0.182
		Europe, alfalfa	7(3)	0.0024	0.0013	2.45**	0.416	0.447
		Europe, clover	17(2)	0.0040	0.0029	1.28	0.285	0.078
380	1429	California	15(5)	0.0021	0.0019	0.29	0.723	0.039
		New York	14(5)	0.0021	0.0016	1.15	0.565	0.555
		Europe, alfalfa	13(3)	0.0022	0.0016	1.27	0.269	0.076
		Europe, clover	16(3)	0.0031	0.0020	2.00*	0.323	0.22

^ANumbers of singleton SNPs indicated in parentheses

* Significant ($P < 0.05$) and ** highly significant ($P < 0.01$) Tajima's D values [43]

for the North American samples and a π value for the European samples for each locus and compare them, 1-sided $P = 0.09$, paired t-test).

All loci possessed indels. We observed two 1 bp indels in locus 41. Locus 870 had a 1 bp, a 2 bp, a 3 bp and a 5 bp indel. Locus CAA showed two 1 bp, a 4 bp and a 6 bp indel. Finally, locus 380 had five 1 bp indels, a 4 bp indel, and a 14 bp indel. There were additional indels that we did not directly observe: multiple individuals had sequencing problems at the same region likely due to a heterozygous indel. These can be inferred from Figure 1.

Tajima's D and Hardy-Weinberg Estimates

Under neutrality, the values of π (based on the number of pairwise differences between individuals) and θ (based on the number of segregating sites) should be equal [42,47]. Tajima's D test compares these two measures of diversity. D values that are significantly different from zero indicate non-neutral evolution resulting from demographic changes or from selection [43]. We calculated Tajima's D for each locus and each population and observed that across all populations and all loci, with only one exception (locus CAA, California), Tajima's D values were positive (Table 2). Five of 16 values were significantly positive at the 95% level. All loci had at least one significant positive D value. Four of the five significant D values were from European populations.

New York and California populations were in Hardy-Weinberg equilibrium for all loci except the autosomal locus 870 (New York: $X^2 = 94.2$, $P = 0.001$, California: $X^2 = 84.2$, $P = 0.014$). Both deviations at locus 870 were towards a deficiency of heterozygotes. All four loci were in Hardy-Weinberg equilibrium within the two European populations.

Population Structure

F_{st} values indicated moderate differentiation between most populations, with an average value of 0.188 (Table 3). The lowest value was observed for the comparison of European alfalfa and European clover populations (0.155) and the highest for the comparison of California and clover-feeding populations from Europe (0.228).

Table 3: F_{st} values for each pairwise population comparison.

	New York	Europe, Alfalfa	Europe, Clover
Europe, Alfalfa	0.199		
Europe, Clover	0.186	0.155	
California	0.198	0.161	0.228

Linkage Disequilibrium

We estimated LD levels, as measured by the correlation between variable bases (r^2), separately for each population and each locus. The average correlation at both short-range (less than 1 kb) and long-range (about 10 kb) distances is listed in Table 2, and the distribution of r^2 within a kilobase for all loci pooled is displayed in Figure 2. Figure 3 illustrates the significance of these pairwise comparisons at both distances. Generally, we observed significant levels of LD within 1 kb distances, with an overall average of 0.38, ranging from 0.19 to 0.72. LD decayed slowly within a kilobase (lines in Figure 2), with the fastest rate of decay observed in the New York population. At the larger scale, average r^2 values were low relative to the shorter spatial scale with an average of 0.20, although some of these comparisons were still significant (Figure 3).

We also examined levels of LD across loci. Between the two autosomal loci, 870 and 41 (it is unknown whether they are on the same chromosome), the average r^2 was 0.15. Loci CAA and 380 on the X chromosome had an interlocus average r^2 of 0.18.

Discussion

Sequence Diversity and Linkage Disequilibrium

We have investigated the levels of nucleotide diversity and LD across four genomic regions in two introduced pea aphid populations, New York and California, and two host races from native populations in Europe. This is the first estimate of short and longer range LD decay for this species. Previous studies using primarily mtDNA sequence variation, RFLPs, and allozymes have reported low diversity in pea aphid populations [32,48-50]. Like these studies, we find low diversity, with π averaging 0.0056 across all loci and all four populations, ranging from 0.0008 (locus CAA, California) to 0.0116 (locus 41, Europe, clover).

The two loci on the X chromosome exhibit roughly half the diversity of the two loci on the autosomes. These lower X chromosome values may result, at least in part, from the lower effective population size for the X chromosome. In a population with equal numbers of males and females there are three X chromosomes for every four autosomes because females are XX and males are XO. Under neutrality, nucleotide diversity is directly proportional to effective population size [51]. However, these X chromosome π values remained lower than the autosomal values even after multiplying by 4/3 to compensate for the X to autosomal population size differences. If this pattern remains consistent across autosomal and X chromosome loci, then this implies that other factors, such as selection, may act differentially on the X chromosome and autosomes [52].

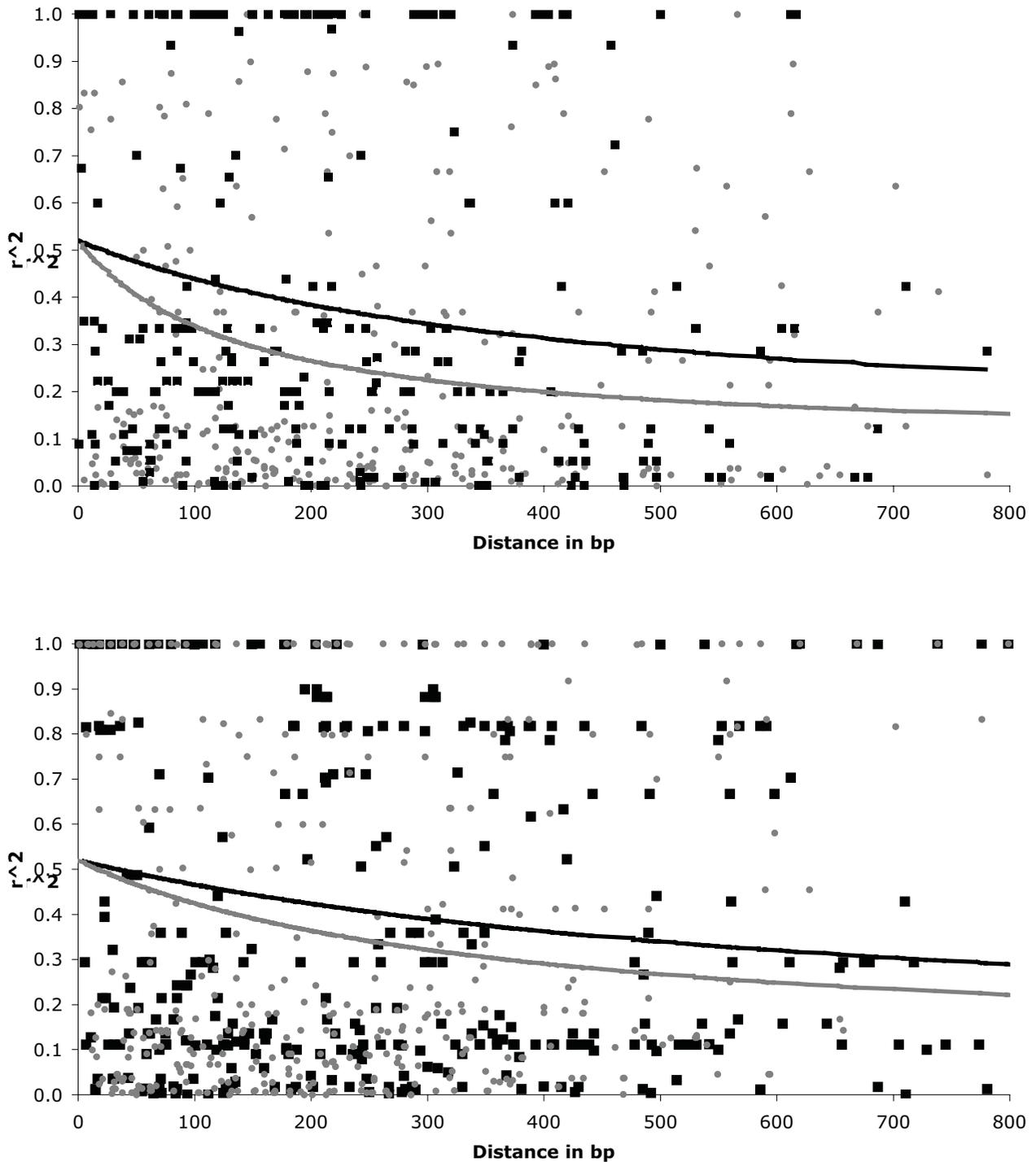


Figure 2
 Plot of the squared correlations (r^2) versus distance in bp between sites for populations from New York (grey circles) and California (black squares) in the top panel, and Europe, clover (grey circles), and Europe, alfalfa (black squares), in the lower panel. Data from all four loci (two sequenced amplicons per locus) are included. The fitted curve of Hill and Weir [39] illustrating the expected decay of LD is shown for each population.

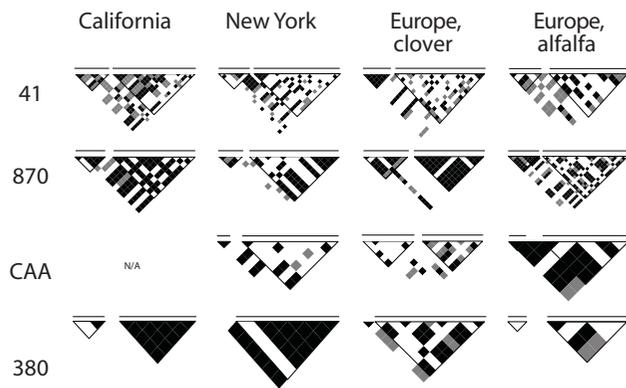


Figure 3
Significance of pairwise LD between different sites.

Each variable site for a particular locus and population is represented along the left diagonal as well as across the top of each triangle. The significance of the pairwise comparison is represented as a diamond where they intersect, with black $P < 0.01$, grey $0.01 < P < 0.05$ and white nonsignificant. Each triangle has a different number of diamonds because each locus and population harbored a different number of variable sites. Black lined inverted triangles indicate comparisons within 1 kb (within one of the sequenced amplicons, thus two triangles in most cases), as do the horizontal lines above each graph. Comparisons across approximately 10 kb (between the two sequenced amplicons at each locus) fall outside of those black lined inverted triangles. No pairwise comparisons were computed for the CAA locus for the California population because there was not sufficient nucleotide variation. Refer to Table 2 for total number of variable sites per locus for each of the populations.

In a previous study [53], we sequenced portions of the coding regions from 27 different loci from an average of ten individuals from disparate geographical locations. Of these 27 loci, 16 exhibited silent site polymorphism. The average diversity of this silent site polymorphism was 0.0053 (ranging from 0.0006 to 0.0114), very similar to the average of 0.0056 we report here for noncoding variation despite low levels of codon bias in the pea aphid [54].

The levels of noncoding DNA diversity in the pea aphid are two and a half times lower than the diversity estimates of 0.0153 for intergenic regions of *Drosophila simulans* [55] and 0.0142 for noncoding regions of *Aedes aegypti* [56]. Although it is possible that the pea aphid has a lower mutation rate, the more likely explanation for this comparatively low diversity is small effective population size. The pea aphid has a large absolute population size during its parthenogenetic phase in the spring and summer months, but many individuals are genetic clones and thus effective population size is much lower. Biased sex ratios [57], high variance in male mating success [58] and low survival of eggs over the winter might cause additional

reductions in effective population size. Finally, as pea aphids are pests on field crops, it is likely that many populations have experienced declines due to pesticides, further reducing the effective population size.

We also examined levels of LD at two scales: within a kilobase, and across approximately 10 kb. Generally, LD is high and decays slowly within a kilobase (Fig. 1). Over ~ 10 kb, LD has declined to levels only slightly higher than LD averages across loci and therefore what we consider background levels of LD. Thus, this limited survey provides no evidence for long range LD in any of the four populations surveyed. This suggests that LD mapping in the pea aphid will require a relatively high density of molecular markers distributed at the scale of about a kilobase.

Native Versus Introduced Populations

Pea aphids were introduced to North America, probably from Europe [26]. They are mild pests on field crops such as alfalfa and clover, and have likely been introduced to North America several times over the last 100 to 200 years via agricultural routes. If only a few individuals were introduced at a time, the North American populations would likely exhibit decreased levels of variation relative to native populations [59]. We therefore hypothesized that we would observe lower levels of variation in North American populations relative to European populations. This does not appear to be the case, as both π and θ are similar between the two regions. Likewise, LD levels are similar in populations from both regions. We conclude that native and introduced populations will not have differential utility in LD mapping efforts.

It is unknown how many times or where the pea aphid was introduced into North America. It is possible that repeated introductions have occurred, supplementing the molecular diversity provided by earlier introductions. Consistent with this model, the introduced populations are not depauperate for nucleotide diversity. Further, Tajima's D values are largely positive which can be indicative of admixture, a reduction in population size, or balancing selection [43]. It seems unlikely that balancing selection is acting on all four of these non-coding loci. We therefore favor the hypothesis that the positive Tajima D values reflect demographic factors.

Host plant specialization

Pea aphids are specialized to a variety of host plants in Europe, including alfalfa and clover [32,33]. Similarly, pea aphids in New York demonstrate strong host plant specialization [23,30], whereas in California both specialists and generalists exist [35]. The specialization in New York either represents introduction of both specialists from the native range, or introduction followed by a sec-

ondary event of specialization. All New York individuals were sampled from alfalfa. F_{st} values between New York and European alfalfa and between New York and European clover populations are similar (average of 0.199 and 0.186, respectively), providing no evidence for the source of the New York populations. Both of these values are higher than for the comparison of European clover and alfalfa specialists ($F_{st} = 0.155$). This value is only slightly higher than the level of F_{st} reported by Simon et al. [32] for comparisons of clover and alfalfa specialists in France either by allozymes ($F_{st} = 0.098$) or microsatellites ($F_{st} = 0.104$).

The California pea aphids examined here were sampled from alfalfa, but all are generalists in that they can feed on both hosts (T. Leonardo, pers. comm). Interestingly, population differentiation is high between California and the European clover clones ($F_{st} = 0.228$), while lower in comparison with the European alfalfa clones ($F_{st} = 0.168$). Although this difference is not statistically significant (one-tailed t-test, $P = 0.19$) the trend of lower differentiation between California and the alfalfa specialists leads us to hypothesize that the host plant generalist aphids found in California are derived from European alfalfa specialists, but that subsequently there has been interbreeding with other introduced populations. Further tests of this hypothesis will require more extensive population sampling and marker development but will provide insight into the interplay of invasion of a new habitat and the gain or loss of host-plant specialization.

Conclusion

The recent release of the of the pea aphid genome sequence will facilitate mapping efforts aimed at identifying loci underlying variable phenotypes unique to this species, such as polyphenisms and host plant specialization. To inform these future studies, here we provide the first systematic study of nucleotide diversity and LD in both native and introduced populations of the pea aphid. Further, we tested and rejected the hypothesis that bottlenecks associated with the importation of pea aphids into North America have resulted in introduced and native populations being valuable for different mapping needs (coarse versus fine-scale mapping, respectively), finding low levels of nucleotide diversity and moderate levels of LD in all of the populations sampled.

Authors' contributions

JAB and DLS conceived of the study, JAB carried out the experiments, JAB and SVN performed the analyses, and all three authors participated in the writing of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We thank J.-C. Simon, T. Leonardo, and C. Braendle for aphid clones and P. Y. Hui for laboratory assistance. Thanks to R. Bickel, T. Leonardo, A. Tarone, T. Turner and two anonymous reviewers for thoughtful comments on the manuscript. The project was supported by Award Number F32GM076962 from the National Institute of General Medical Sciences to JAB and by grants from National Institutes of Health (GM063622-06A1) and National Science Foundation (IOS-0640339) to DLS and a David & Lucile Packard Foundation Fellowship to DLS.

References

1. Long AD, Langley CH: **The power of association studies to detect the contribution of candidate genetic loci to variation in complex traits.** *Genome Res* 1999, **9**:720-731.
2. Gibbs RA, Belmont JW, Hardenbol P, Willis TD, Yu FL, Yang HM, Ch'ang LY, Huang W, Liu B, Shen Y, et al.: **The International Hap-Map Project.** *Nature* 2003, **426**:789-796.
3. Nordborg M, Borevitz JO, Bergelson J, Berry CC, Chory J, Hagenblad J, Kreitman M, Maloof JN, Noyes T, Oefner PJ, et al.: **The extent of linkage disequilibrium in *Arabidopsis thaliana*.** *Nature Genet* 2002, **30**:190-193.
4. Nordborg M, Hu TT, Ishino Y, Jhaveri J, Toomajian C, Zheng H, Bakker E, Calabrese P, Gladstone J, Goyal R, et al.: **The pattern of polymorphism in *Arabidopsis thaliana*.** *PLoS Biol* 2005, **3**:e196.
5. Zhu YL, Song QJ, Hyten DL, Van Tassell CP, Matukumalli LK, Grimm DR, Hyatt SM, Fickus EW, Young ND, Cregan PB: **Single-nucleotide polymorphisms in soybean.** *Genetics* 2003, **163**(3):1123-1134.
6. Liu A, Burke JM: **Patterns of nucleotide diversity in wild and cultivated sunflower.** *Genetics* 2006, **173**(1):321-330.
7. Remington DL, Thornsberry JM, Matsuoka Y, Wilson LM, Whitt SR, Doebley J, Kresovich S, Goodman MM, Buckler ES: **Structure of linkage disequilibrium and phenotypic associations in the maize genome.** *Proc Natl Acad Sci USA* 2001, **98**(20):11479-11484.
8. Tenaillon M, Sawkins MC, Long AD, Gaut RL, Doebley JF, Gaut BS: **Patterns of DNA sequence polymorphism along chromosome 1 of maize (*Zea mays ssp. mays* L.).** *Proc Natl Acad Sci USA* 2001, **98**(16):9161-9166.
9. Reich DE, Cargill M, Bolk S, Ireland J, Sabeti PC, Richter DJ, Lavery T, Kouyoumjian R, Farhadian SF, Ward R, et al.: **Linkage disequilibrium in the human genome.** *Nature* 2001, **411**(6834):199-204.
10. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, et al.: **The structure of haplotype blocks in the human genome.** *Science* 2002, **296**(5576):2225-2229.
11. Backstrom N, Qvarnstrom A, Gustafsson L, Ellegren H: **Levels of linkage disequilibrium in a wild bird population.** *Biology Letters* 2006, **2**:435-438.
12. Cutter AD, Baird SE, Charlesworth D: **High nucleotide polymorphism and rapid decay of linkage disequilibrium in wild populations of *Caenorhabditis remanei*.** *Genetics* 2006, **174**:901-913.
13. Cutter AD: **Nucleotide polymorphism and linkage disequilibrium in wild populations of the partial selfer *Caenorhabditis elegans*.** *Genetics* 2006, **172**:171-184.
14. Andolfatto P, Przeworski M: **A genome-wide departure from the standard neutral model in natural populations of *Drosophila*.** *Genetics* 2000, **156**:257-268.
15. Moran NA: **The evolution of aphid life cycles.** *Ann Rev Ent* 1992, **37**:321-348.
16. Blackman RL: **Reproduction, cytogenetics and development.** In *Aphids: Their biology, natural enemies & control* Volume 2A. Edited by: Minks AK, Harrewijn P. Amsterdam: Elsevier; 1987:163-195.
17. Loxdale HD: **The nature and reality of the aphid clone: genetic variation, adaptation and evolution.** *Agricult Forest Ent* 2008, **10**:81-90.
18. Via S: **Inducing the sexual forms and hatching the eggs of pea aphids.** *Ent Exp Appl* 1992, **65**:119-127.
19. MacKay PA, Reeleder DJ, Lamp RJ: **Sexual morph production by apterous and alate viviparous *Acyrtosiphon pisum* (Harris) (Homoptera: Aphididae).** *Can J Zool* 1983, **61**:952-957.
20. Buchner P: **Endosymbiosis of animals with plant microorganisms.** New York: Interscience Publishers, Inc; 1965.

21. Baumann P, Moran NA, Baumann L: **The evolution and genetics of aphid endosymbionts.** *Bio Science* 1997, **47**:12-20.
22. Sutherland ORW: **The role of crowding in the production of winged forms by two strains of the pea aphid, *Acyrtosiphon pisum*.** *J Insect Phys* 1969, **15**:1385-1410.
23. Via S: **Reproductive isolation between sympatric races of pea aphids. I. Gene flow restriction and habitat choice.** *Evolution* 1999, **53**:1446-1457.
24. Nault LR: **Arthropod transmission of plant viruses: a new synthesis.** *Ann Ent Soc Am* 1997, **90**:521-541.
25. Shingleton AW, Sisk GC, Stern DL: **Diapause in the pea aphid (*Acyrtosiphon pisum*) is a slowing but not a cessation of development.** *BMC Dev Biol* 2003, **3**:7.
26. Thomas C: **A list of the species of the tribe Aphidini, family Aphidae, found in the United States, which have been heretofore named with descriptions of some new species.** *State Lab Nat Hist Bull* 1878, **1**:1-16.
27. Nei M, Maruyama T, Chakraborty R: **The bottleneck effect and genetic variability in populations.** *Evolution* 1975, **29**:1-10.
28. Crow JF, Kimura M: **An Introduction to Population Genetics Theory.** New York: Harper and Row; 1970.
29. Maruyama T, Fuerst PA: **Population bottlenecks and nonequilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck.** *Genetics* 1985, **11**:675-689.
30. Via S, Bouck AC, Skillman S: **Reproductive isolation between divergent races of pea aphids on two hosts. II. Selection against migrants and hybrids in the parental environments.** *Evolution* 2000, **54**:1626-1637.
31. Hawthorne DJ, Via S: **Genetic linkage of ecological specialization and reproductive isolation in pea aphids.** *Nature* 2001, **412**:904-907.
32. Simon JC, Carre S, Boutin M, Prunier-Leterme N, Sabater-Munoz B, Latorre A, Bournoville R: **Host-based divergence in populations of the pea aphid: insights from nuclear markers and the prevalence of facultative symbionts.** *Proc R Soc Lond Ser B* 2003, **270**:1703-1712.
33. Sandstrom J: **Temporal changes in host adaptation in the pea aphid, *Acyrtosiphon pisum*.** *Ecol Entomol* 1996, **21**:52-56.
34. Ferrari J, Godfray CJ, Faulconbridge AS, Prior K, Via S: **Population differentiation and genetic variation in host choice among pea aphids from eight host plant genera.** *Evolution* 2006, **60**:1574-1584.
35. Leonardo TE, Muiru GT: **Facultative symbionts are associated with host plant specialization in pea aphid populations.** *Proceedings: Biological Sciences* 2003, **270**:S209-S212.
36. Braendle C, Caillaud MC, Stern DL: **Genetic mapping of *aphicarus* – a sex-linked locus controlling a wing polymorphism in the pea aphid (*Acyrtosiphon pisum*).** *Heredity* 2005, **94**:435-442.
37. Warnes GR: **The genetics package.** *R News* 2003, **3**:9-13.
38. Team RDC: **R: A language and environment for statistical computing.** 2.3.1 edition. Vienna, Austria: R Foundation for Statistical Computing; 2006.
39. Hill WG, Weir BS: **Variances and covariances of squared linkage disequilibria in finite populations.** *Theor Popul Biol* 1988, **33**:54-78.
40. Inc. SI: **SAS/STAT user's guide. Volume 2.** Cary, NC: SAS Institute, Inc; 1989.
41. Nei M: **Molecular Evolutionary Genetics.** New York: Columbia University Press; 1987.
42. Watterson GA: **On the number of segregating sites in genetical models without recombination.** *Theor Popul Biol* 1975, **7**:256-275.
43. Tajima F: **Statistical method for testing the neutral mutation hypothesis by DNA polymorphism.** *Genetics* 1989, **123**(3):585-595.
44. Raymond M, Rousset F: **GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism.** *J Heredity* 1995, **86**:248-249.
45. Weir BC, Cockerham CC: **Estimating F-statistics for the analysis of population structure.** *Evolution* 1984, **38**:1358-1370.
46. Guo SW, Thompson EA: **Performing the exact test of Hardy-Weinberg proportion for multiple alleles.** *Biometrics* 1992, **48**:359.
47. Tajima F: **Evolutionary relationship of DNA sequences in finite populations.** *Genetics* 1983, **105**:437-460.
48. Barrette RJ, Crease TJ, Hebert PDN, Via S: **Mitochondrial DNA diversity in the pea aphid, *Acyrtosiphon pisum*.** *Genome Biology* 1994, **37**:858-865.
49. Boulding EG: **Molecular evidence against phylogenetically distinct host races of the pea aphid (*Acyrtosiphon pisum*).** *Genome Biology* 1998, **41**:769-775.
50. Birkle LM, Douglas AE: **Low genetic diversity among pea aphid (*Acyrtosiphon pisum*) biotypes of different plant affiliation.** *Heredity* 1999, **82**:605-612.
51. Hartl DL, Clark AG: **Principles of Population Genetics.** Sinauer Associates; 1997.
52. Begun DJ, Whitley P: **Reduced X-linked nucleotide polymorphism in *Drosophila simulans*.** *Proc Nat Acad Sci USA* 2000, **97**:5960-5965.
53. Brisson JA, Nuzhdin SV: **Rarity of males in pea aphids results in mutational decay.** *Science* 2008, **319**:58.
54. Rispe C, Legeai F, Gauthier J-P, Tagu D: **Strong heterogeneity in nucleotide composition and codon bias in the pea aphid (*Acyrtosiphon pisum*) shown by EST-based coding genome reconstruction.** *J Mol Evol* 2007, **65**:413-424.
55. Begun DJ, Holloway AK, Stevens K, Hillier LW, Poh Y, Hahn MW, Nista PM, Jones CD, Kern AD, Dewey CN, et al.: **Population genomics: whole-genome analysis of polymorphism and divergence in *Drosophila simulans*.** *PLoS Biol* 2007, **5**:e310.
56. Morlais I, Severson DW: **Intraspecific DNA variation in nuclear genes of the mosquito *Aedes aegypti*.** *Insect Mol Biol* 2003, **12**:631-639.
57. Foster WA: **Aphid sex ratios.** In *Sex Ratio Handbook: concepts and research methods* Edited by: Hardy I. Cambridge University Press; 2002:254-265.
58. Sack C, Stern DL: **Sex and death in the male pea aphid: *Acyrtosiphon pisum*: the life-history effects of a wing dimorphism.** *J Insect Phys* 2007, **7**:45.
59. Mayr E: **Animal Species and Evolution.** Cambridge: Harvard University Press; 1963.

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp



BioMed Central publishes under the *Creative Commons Attribution License (CCAL)*. Under the *CCAL*, authors retain copyright to the article but users are allowed to download, reprint, distribute and /or copy articles in BioMed Central journals, as long as the original work is properly cited.