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1 **Targeted re-sequencing confirms the importance of chemosensory genes in aphid**
2 **host race differentiation**

3

4 Isobel Eyres^{1§}, Ludovic Duvaux¹, Karim Gharbi³, Rachel Tucker¹, David Hopkins¹, Jean-
5 Christophe Simon², Julia Ferrari^{4*}, Carole M. Smadja^{5*}, Roger K. Butlin^{1*}

6

7 * Joint last authors

8

9 1. Department of Animal and Plant Sciences, University of Sheffield, Sheffield, United
10 Kingdom

11 2. INRA, Institut de Génétique, Environnement et Protection des Plantes, UMR 1349
12 IGEPP, Domaine de la Motte, 35653 Le Rheu Cedex, France

13 3. Edinburgh Genomics, Ashworth Laboratories, University of Edinburgh, Edinburgh,
14 United Kingdom

15 4. Department of Biology, University of York, York, United Kingdom

16 5. Institut des Sciences de l'Evolution (UMR 5554 CNRS-IRD-CIRAD-Université de
17 Montpellier), cc065, place Bataillon, Campus Triolet, Université de Montpellier,
18 34095 Montpellier cedex 05, France

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22 targeted resequencing, genome scan.

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28 [§]Corresponding author

29 Isobel Eyres, Department of Animal and Plant Sciences, Alfred Denny Building,
30 University of Sheffield, Western Bank, Sheffield, S10 2TN, UK. Email:

31 i.eyres@sheffield.ac.uk

32

33 **Abstract**

34

35 Host-associated races of phytophagous insects provide a model for understanding
36 how adaptation to a new environment can lead to reproductive isolation and
37 speciation, ultimately enabling us to connect barriers to gene flow to adaptive
38 causes of divergence. The pea aphid (*Acyrtosiphon pisum*) comprises host-races
39 specialising on legume species, and provides a unique system for examining the early
40 stages of diversification along a gradient of genetic and associated adaptive
41 divergence. As host-choice produces assortative mating, understanding the
42 underlying mechanisms of choice will contribute directly to understanding of
43 speciation. As host-choice in the pea aphid is likely mediated by smell and taste, we
44 use capture sequencing and SNP genotyping to test for the role of chemosensory
45 genes in the divergence between eight host-plant species across the continuum of
46 differentiation and sampled at multiple locations across western Europe. We show
47 high differentiation of chemosensory loci relative to control loci in a broad set of pea
48 aphid races and localities, using a model-free approach based on Principal
49 Component analysis. Olfactory and gustatory receptors form the majority of highly
50 differentiated genes, and include loci that were already identified as outliers in a
51 previous study focusing on the three most closely related host races. Consistent
52 indications that chemosensory genes may be good candidates for local adaptation
53 and barriers to gene flow in the pea aphid open the way to further investigations
54 aiming to understand their impact on gene flow, and to determine their precise
55 functions in response to host plant metabolites.

56 Introduction

57

58 Speciation depends on the evolution of barriers to gene flow, and natural selection is
59 now considered to be an important driver in this process (Kirkpatrick & Ravigné
60 2002; Nosil *et al.* 2002; Via 2009; Nosil 2012); local adaptation can lead to
61 reproductive isolation in the face of gene flow. Contact between populations that
62 have undergone some divergence through selection or geographical isolation is a
63 common occurrence, and the opportunity that this provides for gene flow may cause
64 breakdown of the initial divergence. An important challenge in current speciation
65 research is therefore to understand how lineages can maintain differentiation and
66 progress towards speciation despite ongoing gene exchange (Smadja & Butlin 2011).

67

68 With the exception of polyploidy, speciation tends to be a long process, requiring the
69 progressive buildup of reproductive isolation (Abbott *et al.* 2013). Where lineages
70 are undergoing ecological speciation in the face of gene flow, reproductive isolation
71 can start with the action of divergent selection on locally adaptive loci. This initial
72 divergence may then be facilitated by the association of local adaptation and
73 assortative mating, by close linkage in the genome, by pleiotropy or where the same
74 trait influences both components of isolation (Felsenstein 1981; Servedio 2008;
75 Smadja & Butlin 2011). There is then the possibility that initially divergent genome
76 regions will expand over time as gene flow diminishes between lineages (Feder *et al.*
77 2012).

78

79 One way to study the progress of barriers to gene flow and their role in contributing
80 to speciation is to identify candidate loci in populations experiencing divergence
81 based on local adaptation, early in the process of speciation, and to track the action
82 of selection across a continuum of divergence through space or time (Jones *et al.*
83 2012; Martin *et al.* 2013). In order to do this effectively, we must be able to identify
84 loci involved in the initial local adaptation with confidence. While many studies have
85 identified highly differentiated loci that are potentially under divergent selection,
86 successful follow-up studies to outlier scans have rarely been achieved (Rogers &

87 Bernatchez 2005, 2007; Wood *et al.* 2008; Butlin 2010; Jones *et al.* 2012; Malinsky *et*
88 *al.* 2015).

89

90 Quantitative Trait Locus (QTL) studies have traditionally been used to identify
91 genome regions connected to local adaptation (Hawthorne & Via 2001; Ungerer &
92 Rieseberg 2003; Baxter *et al.* 2008), and population genomic scans for outlier loci are
93 commonly used to identify outlier loci relating to local adaptation and the reduction
94 of gene flow between populations (Nosil *et al.* 2008; Galindo *et al.* 2010). Both of
95 these methods can now be performed with very large numbers of markers, and
96 therefore can have high resolution (Hohenlohe *et al.* 2010). However, it can still be a
97 challenge to pinpoint the specific targets of selection with confidence; there are
98 multiple reasons why outlier loci may be detected in one sample only, and it is
99 important to confirm that identified outliers are the true targets of natural selection.
100 Functional interpretation of differentiated, 'outlier', loci or QTL (Barson *et al.* 2015)
101 and experimental tests for the action of selection (Barrett *et al.* 2008; Gompert *et al.*
102 2012) are ultimately critical but can be a major investment. Given the uncertainties
103 associated with outlier detection (e.g. Hermisson 2009), an important step in many
104 systems is to confirm outliers by repeating analyses in new samples, separated in
105 time or space.

106

107 Where we have good reason to suspect the involvement of a gene category in a
108 speciation system, targeted gene sequencing can allow us to look in more specific
109 regions for signals of reduced gene flow, whilst avoiding some of the problems of the
110 population genomics and QTL methods (e.g. false positives caused by multiple
111 testing, uncertainty about the genuine target of selection) (Smadja *et al.* 2012).

112 Combining QTL mapping with outlier loci scans, to associate outliers with
113 phenotypes, can provide a powerful indication of the source of selection driving
114 speciation (Rogers & Bernatchez 2005; Via & West 2008; Via 2012; Via *et al.* 2012),
115 and reveals the enormous potential we now have to follow up on outlier scans once
116 outliers have been confidently identified.

117

118 Host-race formation in phytophagous insects represents an excellent model for the
119 evolution of reproductive isolation resulting from divergent selection in the face of
120 gene flow (Drès & Mallet 2002; Bush & Butlin 2004; Forister *et al.* 2011). Very high
121 diversity is linked to specialization via host-switching and co-speciation in many
122 insect taxa (Weiblen & Bush 2002). Host races (genetically distinct populations,
123 locally adapted to different host plant species but still experiencing some level of
124 gene flow), and their host plants (clearly defined species, but often geographically
125 proximate), provide a very helpful set-up for examining the interplay between
126 divergent selection and ongoing genetic exchange (Drès & Mallet 2002).

127

128 The pea aphid, *Acyrtosiphon pisum*, is a well-established model for the study of
129 ecological speciation (Peccoud & Simon 2010), and was the first aphid species to
130 have its genome sequenced (The International Aphid Genomics Consortium 2010). *A.*
131 *pisum* lives and feeds on species of the bean family (Fabaceae); in Europe, at least 15
132 genetically distinct host-plant-associated populations (races) have been described,
133 each associated with one or a few host plant species. *A. pisum* races show increased
134 preference for and performance on their associated plant species in comparison to
135 alternative host plants (Via 1991; Ferrari *et al.* 2008). Races form a continuum of
136 divergence ranging from pairs which produce around 10% F1 hybrids up to and
137 including strongly isolated host races with F_{ST} exceeding 0.8 in sympatry, which
138 probably no longer experience gene flow (Peccoud *et al.* 2009, 2015). This
139 continuum of divergence between races provides us with a rare opportunity to
140 examine the progression of barriers to gene flow across the genome. Although pea
141 aphid host plants have overlapping ranges (Peccoud *et al.* 2009), aphid host-races
142 both feed and mate on their specific plants, which leads to assortative mating and
143 the potential for the evolution of reproductive isolation.

144

145 Because assortative mating is related to host-plant, how aphids select a plant to
146 settle and feed on has the potential to be an important component in the evolution
147 of premating isolation. Indeed, the chemosensory system has frequently contributed
148 to host, habitat and mate choice in a range of study systems (reviewed in Smadja &
149 Butlin 2008). Aphid recognition of the host plant and establishment of phloem

150 feeding has several stages (Powell *et al.* 2006; Simon *et al.* 2015); before an aphid
151 settles to feed it may respond to plant volatiles near the surface of the leaf
152 (Nottingham & Hardie 1993), and undertake initial probing with the stylets (Caillaud
153 & Via 2000). Volatile and non-volatile odor and taste molecules are recognized in
154 insects by a set of chemoreceptors found in the chemosensory organs (antennae,
155 mouth parts, and maxillary palps) (Kopp *et al.* 2008; Shiao *et al.* 2013). These
156 chemosensory genes include gustatory (GR), odorant (OR) and ionotropic (IR)
157 receptors (Hallem *et al.* 2006; Croset *et al.* 2010), as well as odorant binding proteins
158 (OBPs) which are involved in the transport of odorants (Leal 2005), chemosensory
159 proteins (CSPs) and sensory neuron membrane proteins (SNMPs) (Leal 2005; Jin *et*
160 *al.* 2008; Vogt *et al.* 2009). Evidence is accumulating for the key role of
161 chemosensory genes in host specialization in insects (Visser 1986; Whiteman &
162 Pierce 2008; Schymura *et al.* 2010). They exist in large multigene families in most
163 insects (Sánchez-Gracia *et al.* 2009), and both their birth and death mode of
164 evolution and the detection of positive selection on branches of these multi-gene
165 families point to rapid evolution in specialized lineages (Matsuo 2008; Briscoe *et al.*
166 2013; Duvaux *et al.* 2015).

167

168 In the pea aphid, multiple lines of evidence now point to the importance of
169 chemosensory genes as a category in underpinning feeding decisions. Behavioural
170 studies indicate that aphids show a distinct preference for their associated host plant
171 when presented with a choice of alternative hosts (Ferrari *et al.* 2006), as well as
172 increased survival and fecundity. Genetic evidence from whole genome scans
173 (Jaquiéry *et al.* 2012), targeted re-sequencing (Smadja *et al.* 2012), examination of
174 copy number variation (Duvaux *et al.* 2015) and gene expression (Eyres *et al.* 2016)
175 have all found indications that chemosensory genes differ between pea aphid races.
176 Although these studies confirm the value of further investigation of chemosensory
177 genes in pea aphids, and provide us with a set of potentially interesting target
178 chemosensory genes, this type of broad genomic study is prone to problems of false
179 positives, as well as questionable reliability and repeatability (François *et al.* 2016;
180 Jensen *et al.* 2016). Before we progress to examine target genes in more detail, it is
181 important to confirm the findings of these studies.

182

183 There is a large number of tests available for the detection of outliers relating to
184 local adaptation (e.g. Beaumont & Nichols 1996; Beaumont & Balding 2004; Foll &
185 Gaggiotti 2008; Whitlock & Lotterhos 2015). In general these methods evaluate the
186 genetic differentiation between populations and identify extreme values
187 corresponding to candidate regions of the genome. Outlier scans have proved
188 successful in many cases at identifying loci potentially under selection (Nosil *et al.*
189 2009; Butlin 2010). However, a disadvantage of many outlier detection methods is
190 their requirement for *a priori* assignment of individuals to populations (Yang *et al.*
191 2012; François *et al.* 2016). In populations undergoing divergence in the face of gene
192 flow, such as pea aphid host-associated races, the potential for sampling migrants
193 and hybrids is high, making confident assignment of individuals to populations a
194 difficult requirement to fulfill. In this study we use PCAdapt (Duforet-Frebourg *et al.*
195 2014, 2015), a method for the detection of candidate loci using Principal
196 Components analysis (PCA), which is individual-based and therefore is well suited to
197 analysing data where population level assignment of individuals is uncertain. As our
198 interest lies in identifying loci relating to differences in host-plant preference
199 between aphids, rather than in analysing genetic population structure, it is useful to
200 be able to identify outliers based on genetic divergence rather than *a priori*
201 population classification. Because PCAdapt identifies factors underlying the major
202 axes of genetic variation among individuals, and then searches for loci strongly
203 influencing these factors, it also allows us to examine only the important variation
204 among races, rather than all pairwise race comparisons, thus reducing the risk of
205 false positives from multiple comparisons. Furthermore, unlike many model-based
206 outlier methods, PCAdapt does not assume an island model, and so is better suited
207 to the wide range of levels of differentiation seen among pea aphid races.

208

209 Previous work (Smadja *et al.* 2012) has identified chemosensory genes as a
210 promising set of candidate barrier loci; in an F_{ST} outlier scan of 9889 SNPs in 172
211 target genes (chemosensory and control) the proportion of outlier SNPs identified in
212 Grs and Ors was significantly higher than in non-chemosensory control genes.
213 Furthermore, this study identified a set of 18 chemosensory genes that were

214 unusually divergent between host races. These chemosensory candidates were
215 identified as outliers in comparisons between three of the more closely related,
216 although still highly specialized, pea aphid races (feeding on *Medicago sativa*,
217 *Trifolium pratense*, and *Lotus pedunculatus*) (Ferrari *et al.* 2008, 2012; Peccoud *et al.*
218 2009) in a single geographic region.

219

220 In diverging populations, alleles underlying local adaptation can differ among
221 localities because of drift, availability of mutations or differences in selection, but
222 repeated patterns of differentiation across the geographic range of the pea aphid
223 races would provide evidence for loci that diverge in response to common divergent
224 selection pressures rather than as the result of stochastic processes. In addition, we
225 wished to test whether loci involved in differentiation between one pair of races,
226 were also likely to contribute to differentiation between other pairs. Therefore, our
227 intention here was to test the pattern of divergence in chemosensory genes across
228 (a) a larger number of pea aphid races along the continuum of differentiation and (b)
229 multiple populations covering a broader geographic distribution. Incorporating a
230 wider selection of aphid races, including the far more divergent races associated
231 with *Lathyrus pratensis*, *Cytisus scoparius* and *Ononis spinosa*, will ultimately allow
232 us to capitalize on the continuum of divergence in pea aphids, by examining patterns
233 relating to the extent of divergence between races and the progression of barriers to
234 gene flow across the genome. Additional races also potentially facilitate the
235 identification of new chemosensory outliers relating to local adaptation in previously
236 untested races. Repeating outlier scans on independently sampled aphids allows us
237 to exclude false positives from the initial scan, and confirm the association of outliers
238 with host race, the target environmental variable. As argued above, this
239 confirmation is likely to be a valuable step in many comparable studies.

240 **Materials and Methods**

241

242 *SNP data from Capture Sequencing*

243

244 We used the capture sequencing dataset generated in Duvaux *et al.* (2015) using
245 SureSelect. This was generated from 120 aphids (between 12 and 17 individuals per
246 host plant) from eight host plant species (*Lotus pedunculatus*, *Lotus corniculatus*,
247 *Medicago sativa*, *Trifolium pratense*, *Lathyrus pratensis*, *Pisum sativum*, *Cytisus*
248 *scoparius* and *Ononis spinosa*), sampled 30 m apart to ensure distinct genotypes
249 (supplementary file 1a). Aphids were collected in south-east England over three
250 years, all less than 100km apart. SureSelect, which uses RNA probes to capture
251 regions of interest from genomic DNA, was used prior to sequencing. Capture targets
252 were candidate genes potentially relating to identification and selection of host
253 plants, including all of the chemosensory genes that had been partially or fully
254 annotated in Assembly 1.0 of the pea aphid genome (Smadja *et al.* 2009; Zhou *et al.*
255 2010): 79 olfactory receptor (Or) genes, 77 gustatory receptor (Gr) genes, 11 odorant
256 binding protein (OBP) genes and 10 chemosensory protein (CSP) genes. Other genes
257 potentially involved in chemosensation, 11 ionotropic glutamate receptor (Ir) genes
258 and 9 sensory neuron membrane protein (SNMP) genes, were also included as
259 targets, along with putative cis-regulatory regions relating to all these genes (for Ors,
260 Grs, OBPs and CSPs, 50bp predicted regions were identified upstream of target
261 genes and for IRs and SNMPs, 500 bp upstream regions were targeted, details in
262 Duvaux *et al.* (2015)). 69 genes from the P450 gene family (Zhang *et al.* 2010)
263 potentially relating to detoxification, five pheromone synthesis genes, and 5 salivary
264 protein genes were also included. 211 randomly chosen genes were added as
265 controls. After mapping reads to the pea aphid genome (assembly 2.1) using Stampy
266 1.0.17, 21610 SNPs were called for all 120 aphid genotypes using Platypus 0.7.9.2
267 (Rimmer *et al.* 2014).

268

269

270 *PCAdapt analysis of capture sequencing data*

271

272 Capture data were filtered, removing SNPs based on the following criteria: quality
273 score < 40, copy number != 1, minor allele count < 3, scored in < 60% of individuals,
274 observed heterozygotes >10 more than expected. Removing three individuals
275 without copy number information (Med210, Lped84, Lped82), along with duplicate
276 clone Pisum5, left 7232 loci in 116 individuals.

277

278 These 7232 SNPs were analysed using the rapid, PCA-based method in PCAdapt
279 (version 3.0 in R version 3.2.4) (Duforet-Frebourg *et al.* 2015). PCAdapt performs
280 scans for natural selection using Principal Components analysis; examining
281 correlations between SNPs and each principal component allows the detection of
282 SNPs that strongly influence patterns of variation and are putatively involved in
283 adaptive differentiation along these axes. PCAdapt does not require any prior
284 definition of populations.

285

286 An initial run with K=20 principal components was used to select the correct K; a
287 scree plot indicated K=7 (supplementary file 1b) as appropriate. After running with
288 K=7, it was apparent that aphids in the *Lathyrus pratensis*-associated race have a
289 large number of very influential SNPs in PC1 (supplementary file 1c and 1d); as this
290 makes outlier identification difficult, we excluded *Lathyrus*-associated individuals
291 from subsequent analyses. Excluding *Lathyrus*-associated individuals left 104
292 genotypes in 7 races. We then re-ran PCAdapt with one fewer principal component
293 (K=6) (supplementary file 1e). Component-wise outlier scans were performed in
294 PCAdapt, using loadings as the test statistic (corresponding to the correlation
295 between each SNP and the principal component of interest), *p*-values were
296 calculated based on making a Gaussian approximation for each PC and estimating
297 the standard deviation of the null distribution, see Duforet-Frebourg *et al.* (2015),
298 and after converting *p*-values to *q*-values, SNPs with *q*≤0.05 were considered
299 “outliers”. If outliers are randomly distributed in the genome, as might be expected
300 for false positives, the number in any one gene will follow a Poisson distribution. The
301 `poisson.test()` function in R was used to identify “outlier” genes containing
302 significantly more SNPs with *p*≤0.05 than expected by chance, given the overall
303 proportion of outliers and the total number of SNPs per gene, for each principal

304 component in turn (the same strategy as used by Smadja *et al.* 2012). Loci with few
305 SNPs but with a high proportion of outliers may not depart significantly from the
306 Poisson expectation. Therefore, this test may be prone to false-negatives but it is
307 expected to provide a conservative list of genes with strong differentiation.

308

309

310 *Aphid collection and DNA extraction for GoldenGate SNP genotyping*

311

312 Pea aphids were collected from the same eight host-plants as used in the capture
313 sequencing dataset: *La. pratensis*, *O. spinosa*, *C. scoparius*, *Lo. corniculatus*, *Lo.*
314 *pedunculatus*, *P. sativum*, *M. sativa* and *T. pratense*. In the UK, collection took place
315 over two years (2012 and 2013) in locations near Bristol, Peterborough, Sheffield and
316 the Blankney estate in Lincolnshire. Aphids from mainland Europe were collected in
317 France (Mirecourt, Volgesheim, Ranspach and Bugey) and Switzerland. Where
318 possible aphids were included from at least two UK locations and two locations in
319 mainland Europe. In total 29 location-and-race specific groups of aphids were
320 included, with a minimum of two and a maximum of seven sampling locations per
321 race, and a mean of 13.5 individuals per race per sampling location. Details of
322 sampling locations can be found in supplementary files 1f and 1g.

323

324 Aphids were grown up clonally from field-collected individuals on *Vicia faba* in the
325 laboratory to provide enough individuals for DNA extraction. Aphids were stored in
326 ethanol prior to DNA extraction. DNA was extracted from 5 aphids per genotype,
327 using NucleoSpin Tissue Kit standard protocol (Macherey-Nagel, Düren, Germany).

328

329

330 *GoldenGate SNP assay design, sample processing and allele calling*

331

332 Target SNPs were identified in control, chemosensory and detoxification genes in the
333 capture sequencing dataset. To design our custom set of 384 SNPs, flanking
334 sequences of 100bp to either side of target SNPs were processed using the Illumina
335 Assay Design Tool (ADT) in order to confirm their suitability for the assay, and the

336 finalized panel of 384 SNPs was ordered from Illumina (Illumina, San Diego, CA, USA).
337 The final 384 SNPs comprised 222 in chemosensory genes, 71 in non-chemosensory
338 genes of interest (P450s, PS and Rad51C), and 91 in control genes. 127 target SNPs
339 were in genes identified as having a significant excess of outlier SNPs in the capture
340 sequencing dataset. SNP IDs, chromosome positions, and flanking sequences for the
341 panel of 384 SNPs are available in supplementary file 2.

342

343 SNP data were analysed from each plate in turn using the Genotyping module of
344 Illumina's GenomeStudio package (Illumina, San Diego, CA, USA). SNPs were filtered
345 for quality using standard thresholds, SNPs with no polymorphism, SNPs with no
346 heterozygotes, and SNPs with indication of copy number variation were also
347 removed. This left 179 high quality SNPs for further analysis (supplementary file 3).
348 Aphids with more than 12 null SNP calls were removed from the dataset, leaving
349 data for 373 aphids.

350

351

352 *PCAdapt analysis of GoldenGate SNP genotyping data*

353

354 The 179 SNPs in 373 aphids were analysed using the rapid, PCA-based method in
355 PCAdapt (version 3.0 in R version 3.2.4) (Duforet-Frebourg *et al.* 2015). As with the
356 capture sequencing data, an initial run (K=20 principal components) indicated K=7 as
357 appropriate (supplementary file 1h). As with the capture sequencing analysis, *La.*
358 *pratensis*-associated individuals had a large number of influential SNPs in PC1, so
359 were excluded from subsequent analyses (supplementary file 1i).

360

361 Excluding individuals from the *Lathyrus*-associated race (PC1 score > 0.1) left 338
362 genotypes in 7 races. We then re-ran PCAdapt with one fewer principal component
363 (K=6) (supplementary file 1j). Component-wise outlier scans were performed in
364 PCAdapt, using loadings as the test statistic (corresponding to the correlation
365 between each SNP and the principal component of interest), and after converting *p*-
366 values to *q*-values significant SNPs (*q* < 0.05) were considered "outlier SNPs".

367

368

369 *Comparing within and between race variation in GoldenGate SNP genotyping data*

370

371 Based on clustering of aphid genotypes in PCAdapt, the race of 10 aphids was re-
372 assigned from the collection host to the typical host of the genetic cluster to which
373 they belonged (presumed migrants sampled on non-host plants), and six aphids were
374 removed as potential early-generation hybrid individuals (details in supplementary
375 file 1k). We then used locus-by-locus analysis of molecular variance (AMOVA),
376 performed in Arlequin (v 3.5) (Excoffier & Lischer 2010), to examine hierarchical
377 genetic structuring, by race and by locality within race, at each of the 179
378 GoldenGate SNP genotyping loci.

379 **Results**

380

381 *PCAdapt analysis of capture sequencing data: clustering of individuals*

382

383 Running PCAdapt on the capture sequencing dataset without *La. pratensis*
384 individuals, with K=6, allowed us to define six principal axes of variation (figure 1).
385 The first principal component separates the *O. spinosa*-associated individuals in one
386 direction, and (to a lesser extent) *C. scoparius*-associated individuals in the other,
387 from all other races. PC2 separates *C. scoparius* and *O. spinosa*-associated individuals
388 from each other and from all other races. PC3 maintains the three most closely
389 related races in our sample (*T. pratense*, *P. sativum* and *M. sativa*) in a single group,
390 and separates *Lo. pedunculatus*, *O. spinosa* & *C. scoparius*, and *Lo. corniculatus*-
391 associated clusters. PC4 separates both *Lotus*-associated aphid races (*Lo.*
392 *corniculatus* and *Lo. pedunculatus*) from all others. PC5 separates half of the *P.*
393 *sativum*-associated individuals from the other races, and PC6 separates half of the *T.*
394 *pratense*-associated individuals from all other races. *M. sativa*-associated aphids are
395 slightly separated from others in both PC5 and PC6. On the basis of these six axes we
396 can therefore distinguish aphids from the more divergent races, as well as some
397 individuals (but not all) from the two highly similar races of *T. pratense* and *P.*
398 *sativum*.

399

400 F_{ST} distributions (supplementary file 1m), calculated according to groupings defined
401 by the *La. pratensis*-associated principal component and by the other six principal
402 components (PC1-6), showed a large number of SNPs with $F_{ST} = 1$ in both the *La.*
403 *pratensis* and the *C. scoparius*-associated axes of variation, in agreement with
404 PCAdapt findings. As expected, mean F_{ST} was lower in later principal components.
405 The later principal components, which separate the *Medicago*, *Trifolium* and *Pisum*-
406 associated races studied by Smadja et al. (2012) show F_{ST} distributions compatible
407 with the values previously reported (multilocus $F_{ST} = 0.019-0.084$).

408

409

410 *PCAdapt analysis of capture data: outlier analysis*

411

412 Component-wise outlier scans were performed in PCAdapt, using loadings as the test
413 statistic (corresponding to the correlation between each SNP and the principal
414 component of interest), p -values were converted to q -values to control for false
415 discovery rate, and SNPs with $q \leq 0.05$ were considered “outlier SNPs”. A total of
416 503, 557, 299, 274, 111 and 5 SNPs with $q \leq 0.05$ were identified in PC1 to PC6,
417 respectively. These correspond to SNPs with the highest loadings in each
418 component, i.e. they are the most influential SNPs in each axis of variation. Loadings,
419 p -values and q -values of SNPs can be found in supplementary file 4, tab 1. A
420 significantly higher proportion of outlier SNPs were in chemosensory genes than in
421 non-chemosensory genes in principal components 1-4 (z-tests for equality of
422 proportions: PC1 control=0.138, chemosensory=0.168, $p=0.018$; PC2 control=0.147,
423 chemosensory=0.192, $p=0.001$; PC3 control=0.068, chemosensory=0.127, $p=2e-07$;
424 PC4 control=0.072, chemosensory=0.101, $p=0.004$). 614 significant global outliers
425 were identified using the Mahalanobis distance (q -value ≤ 0.05), and again a
426 significantly higher proportion of outlier SNPs were in chemosensory genes than in
427 non-chemosensory genes (control=0.160, chemosensory=0.248, $p=1e-09$).

428

429 For each principal component in turn, and in the global analysis, genes were then
430 considered “outlier genes” when they contained significantly more SNPs with
431 $q \leq 0.05$ than expected by chance (Poisson test), giving: 25 outlier genes in PC1, 24 in
432 PC2, 35 in PC3, 29 in PC4, 15 in PC5 and 5 in PC6, of which 11, 14, 26, 17, 8 and 3,
433 respectively, were chemosensory (figure 2; supplementary file 4, tab 2). 35 outlier
434 genes were identified in the global analysis, of which 18 were chemosensory. Outlier
435 counts and significance test values for all genes can be found in supplementary table
436 4, tab 2. The majority of chemosensory outlier genes identified in each principal
437 component were receptor genes.

438

439 Chemosensory outlier genes tended to be identified in blocks of close similarity and
440 physical distance (figure 2), for example gustatory receptors Gr1-Gr4 (all present on
441 scaffold GL350420) are all outliers in PC3, and where putative promoters were also
442 identified they were often present as outliers along with their downstream gene, for

443 example Or18 and an Or18 putative promoter region are both outliers in PC3, and
444 Gr8 and Gr45 are both outliers in PC4 along with their putative promoter regions.
445 Positioning of scaffolds on a linkage map would provide a more robust
446 understanding of the proximity of these outlier genes in the genome. Of the 18
447 outlier genes identified by Smadja *et al.* (2012) ($p < 0.05$, 3 or more outlier SNPs per
448 gene), 14 were present in the filtered capture sequencing dataset, and nine were
449 confirmed as outliers in this new eight-race comparison (Or17, Or18, Or20, Or21,
450 Or36, Gr8, Gr20, Gr45 and Gr47), along with Gr15 ($p < 0.05$ but with < 3 outlier
451 SNPs).

452

453

454 *SNP data from GoldenGate SNP genotyping*

455

456 After removing low quality SNPs and individuals (see methods), we were left with
457 391 unique aphid genotypes sampled from eight host plants, from between two and
458 seven sampling locations per race, distributed across the UK, France and Switzerland.
459 The retained set of 179 SNPs included 10 in SNMP genes (4 genes), 46 control SNPs
460 (44 genes), 43 in Grs (22 genes), 9 in IRs (3 genes), 31 in Ors (23 genes), 34 in P450
461 genes (24 genes), 1 in a CSP gene, 1 in an OBP gene, 2 in a PS gene and 2 in Rad51C,
462 a control gene identified as an outlier by Smadja *et al.* (2012) (details in
463 supplementary file 3).

464

465

466 *PCAdapt analysis of GoldenGate SNP genotyping data: clustering of individuals*

467

468 Running PCAdapt on the GoldenGate SNP genotyping dataset after excluding *Lo.*
469 *pratensis*-associated individuals, with $K=6$, allowed us to define six principal axes of
470 variation (figure 3). The first principal component separates half of the *Lo.*
471 *corniculatus* individuals in one direction, and the *C. scoparius*-associated individuals
472 in the other direction, from all other races. PC2 separates *O. spinosa*-associated
473 individuals in one direction, and half of the *Lo. corniculatus* individuals in the other
474 direction, from all other races. PC3 separates *O. spinosa*-, *Lo. corniculatus*- and *C.*

475 *scoparius*-associated individuals in one direction, and *P. sativum*-associated
476 individuals in the other, from all other races. PC4 separates the *Lo. pedunculatus*
477 race from all others. *T. pratense* and *M. sativa*-associated individuals consistently
478 have the most negative values in axis 5, and are separated from the other races in
479 opposing directions in PC6.

480

481 Apart from individuals sampled from *Lo. corniculatus*, which broadly split into two
482 clusters based on whether they were sampled in the UK or in mainland Europe on all
483 axes (supplementary figure 1l, figure 3), individuals tend to fall into groups on the
484 basis of host-plant association and not on the basis of geography. A number of
485 individuals in the GoldenGate SNP genotyping dataset appeared to be migrants, i.e.
486 they were collected on one plant species, but are genetically most similar to aphids
487 collected from a different host (e.g. two individuals sampled on *La. pratensis* cluster
488 with other races, one with *Lo. corniculatus*-associated individuals, and one with *P.*
489 *sativum*-associated individuals). A number of individuals may also be hybrids
490 between two races, as they fall into different host-associated clusters on different
491 axes of variation (i.e. they have some SNP alleles typical of one race and other SNP
492 alleles typical of a different race, e.g. one individual collected from *C. scoparius*
493 clusters firmly with *O. spinosa*-associated individuals in PC4). Although aphids from
494 *T. pratense* and *M. sativa*, two of the most closely related races, are not so discretely
495 separated, all other races form distinguishable clusters on at least one principal
496 component.

497

498

499 *PCAdapt analysis of GoldenGate SNP genotyping data: outlier analysis*

500

501 Component-wise outlier scans were performed in PCAdapt, using loadings as the test
502 statistic (corresponding to the correlation between each SNP and the principal
503 component of interest), *p*-values were converted to *q*-values, and SNPs with $q \leq 0.05$
504 were considered “outlier SNPs”. A total of 14, 17, 16, 5, 2 and 1 SNPs with $q \leq 0.05$
505 were identified in PC1 to PC6 respectively. These correspond to SNPs with the
506 highest loadings in each component, and are the influential SNPs in each axis of

507 variation. Loadings, *p*-values and *q*-values of SNPs can be found in supplementary file
508 3. Of these 55 outlier SNPs, 42 (76%) are in chemosensory genes, while only three
509 (5%) are in control genes.

510

511

512 *Arlequin analysis of GoldenGate SNP genotyping data*

513

514 The AMOVA analysis revealed that a large percentage of total genetic variation was
515 between the 8 host-associated races (47.79%, $p < 0.005$), while a much smaller
516 percentage of total variation was attributable to between-locality differences within
517 each race (5.63%, $p < 0.005$). Examining the mean percentage of total genetic
518 variation explained by among-group and between-geographical-location variation in
519 the locus-by-locus analysis allowed us to compare chemosensory and control SNPs.
520 Among-group variation was lower (20.88%) and between-location variation was
521 higher (8.18%) in control loci in comparison to chemosensory loci (45.80% and
522 6.90%, respectively), demonstrating the importance of between race differences in
523 chemosensory genes in comparison to neutral loci.

524

525

526 *Comparison of capture sequencing and SNP genotyping results*

527

528 The capture sequencing dataset contained far more SNPs (7232), examined in fewer
529 individuals (116) in eight races sampled in close proximity, whilst the GoldenGate
530 SNP genotyping dataset contained fewer SNPs (179), examined in a larger number of
531 individuals (391) and covering multiple populations from a far larger European
532 sampling distribution. Nevertheless, the axes identified in our capture sequencing
533 and SNP genotyping datasets are broadly equivalent, although the order differs
534 between analyses (as might be expected from the different composition of the
535 samples): PC1 in the capture sequencing analysis and PC2 in the GoldenGate SNP
536 genotyping analysis both distinguish *O. spinosa*-associated individuals, PC2 in the
537 capture sequencing and PC1 in the GoldenGate SNP genotyping analysis both
538 distinguish *C. scoparius*-associated individuals, while PC3 in the capture sequencing

539 and PC1 in the GoldenGate SNP genotyping analysis both distinguish *Lo. corniculatus*
540 individuals. PC4 separates the *Lo. pedunculatus*-associated population in both
541 analyses, and *M. sativa* and *T. pratense*-associated individuals are distinguished by
542 PC5 in both analyses. *P. sativum*-associated individuals can be distinguished from
543 other races in capture sequencing PC5 and GoldenGate SNP genotyping PC3.

544

545 SNPs with a high loading in their significant GoldenGate SNP genotyping component
546 often have a high loading in the equivalent capture sequencing component; for
547 example, the same SNP (Or36.1_17759) has the top loading in PC4 in both capture
548 sequencing and SNP genotyping analyses, and the top SNP in the capture sequencing
549 PC5 (Gr21.1_461003) has the 11th highest loading in GoldenGate SNP genotyping
550 PC5. Of the 27 significant GoldenGate SNP genotyping outlier SNPs present in the
551 capture sequencing dataset, 23 also make a significant contribution to a capture
552 sequencing factor. The four SNPs not contributing include one control SNP
553 (Control_g84.3_29958), two SNPs in P450 genes (P450_g33.1_53017 and
554 P450_g48.9_38206), and one Gustatory Receptor SNP (Gr1.2_96172). To simulate a
555 null expectation for overlap between the two datasets, the total number of
556 significant GoldenGate SNPs (34) was randomly re-assigned to the set of 179
557 GoldenGate SNPs with 100,000 permutations, and for each permutation we
558 calculated the overlap between capture SNP significance at each SNP and the
559 randomly assigned significant GoldenGate SNP. The real overlap of 23 SNPs
560 significant in both datasets was significantly greater than this expectation ($p <$
561 0.0001).

562

563 There were significant, strong positive correlations between squared loadings of
564 SNPs in the two datasets (figure 4), both when comparing broadly equivalent
565 components (see figure 4), and when looking at maximum loadings per SNP across
566 axes in each dataset (Pearson's correlation = 0.52, $p < 0.0001$).

567

568 There is substantial overlap in the genes identified between axes of variation: 389
569 outlier SNPs are related to more than one principal component in the capture
570 sequencing dataset. Furthermore, 14 chemosensory outlier genes (Poisson test

571 $p < 0.05$) and 5 putative chemosensory promoter outliers (Poisson test $p < 0.05$) are
572 identified in more than one principal component. In the GoldenGate SNP genotyping
573 dataset, 12 outlier SNPs are present in more than one axis of variation.

574 **Discussion**

575

576 Pea aphids provide a promising system for examining the process of speciation with
577 gene-flow, and the progression from initial natural selection acting on adaptive loci
578 to complete genomic differentiation and reproductive isolation between races.

579 Chemosensory genes appear to be important targets of natural selection in this
580 system; examining the differentiation of these genes between races, and how this
581 changes as divergence between races increases, will enable follow up work looking
582 at the genetic architecture of speciation with gene-flow (making good use of the
583 continuum of divergence between races seen in the pea aphid). Previous studies
584 have indicated the value of further investigation of chemosensory genes in pea
585 aphids. However, the types of large-scale genomic study used to identify targets of
586 natural selection are prone to false positives, and have questionable reliability and
587 repeatability (François *et al.* 2016; Jensen *et al.* 2016). Before we progress to
588 examine target genes in more detail, it is important to confirm the findings of these
589 studies. Here, we have undertaken a comprehensive follow-up to previous work,
590 over a broader geographical range than previously examined to confirm the
591 presence of chemosensory outlier genes and their relationship to host-plant
592 adaptation. This is a step that could usefully be applied in many other comparable
593 systems. We have also incorporated additional, more divergent host races giving
594 insight into the role of the same genes at different stages in differentiation.

595

596 We have analysed genetic information from two datasets not previously used to
597 detect outlier SNPs. The capture sequencing dataset (Duvaux *et al.* 2015) included
598 pea aphids from eight races: the three originally looked at by Smadja *et al.* (2012),
599 and five more. As well as confirming the repeatability of outliers among the original
600 three races (table 1), extending the outlier scans to additional races allowed us to
601 test whether the same chemosensory loci were implicated in multiple host shifts.
602 The GoldenGate SNP genotyping dataset included pea aphids from the same eight
603 races, this time sampled from locations across the UK, France and Switzerland.
604 Sampling aphids from more localities across a broader geographical range enabled
605 us to check that outlier genes relate directly to host plant species: there will have

606 been other environmental variables correlated with race where only single
607 geographic regions were examined, whereas replication across different localities
608 and years tends to confirm the relationship between chemosensory gene differences
609 and adaptation to host plants. Given ongoing gene flow among races (Peccoud &
610 Simon 2010), consistent patterns of differentiation are unlikely to be explained by
611 genomic regions of low recombination, whose effect on differentiation is greatest
612 where gene flow is low or absent, but additional evidence for the action of divergent
613 selection is still desirable (Jensen *et al.* 2016). Our results identify good targets for
614 this future work.

615

616 *Chemosensory genes confirmed as targets of selection*

617 We were able to confirm the findings of (Smadja *et al.* 2012), that a significantly
618 higher proportion of outlier SNPs lie in chemosensory genes than in control genes,
619 and that this is true in different samples of aphids, from more races and localities. In
620 both datasets analysed here we again show that Gr and Or genes form the majority
621 of chemosensory outlier loci. We specifically re-identify ten of the outlier genes
622 found in Smadja *et al.* (2012) in the capture sequencing dataset (table 1;
623 supplementary file 6), and three genes (Gr15, Or21 and Or36) were identified in the
624 Smadja *et al.* (2012) analysis and in both the capture sequencing and GoldenGate
625 SNP genotyping datasets. The correlation between the two analyses undertaken in
626 this study was also strong; all chemosensory outlier SNPs identified in the
627 GoldenGate SNP genotyping analysis (incorporating multiple populations per race),
628 that were present in the capture sequencing dataset, were also identified as outliers
629 there.

630

631 By repeating outlier analyses on eight races we confirm that differences in
632 chemosensory genes are important to the divergence of the broader spectrum of
633 pea aphid races, and incorporating more localities in our GoldenGate SNP
634 genotyping dataset allowed us to confirm a direct link between plant choice and
635 chemosensory differences, distinct from other environmental variables that might be
636 correlated with differences between single populations. AMOVA showed large
637 contributions of race and small contributions of locality to genetic variation, a

638 pattern that was more pronounced in chemosensory than in control genes,
639 supporting the relationship between chemosensory gene divergence and race in the
640 face of gene flow. The congruence we observed between multiple independent
641 samples of aphids provides support for the individual outlier genes identified, the
642 general importance of chemosensory genes in between race differences, and more
643 specifically the potential role of Grs and Ors. Although the repeated identification of
644 specific outlier loci could relate to underlying genomic architecture at these sites
645 (Jensen *et al.* 2016) (which one would expect to be the same between aphids in
646 different datasets), comparisons of gene categories are particularly informative
647 indications of the validity of our results as there is little reason to expect that all
648 chemosensory receptor genes will share an unusual feature such as a distinct
649 mutation rate or low diversity, given that they are widely distributed in the genome.
650
651 ORs and GRs in insects tend to be activated in combinations to signal the presence of
652 specific compounds (Hallem *et al.* 2006). As previously suggested (Smadja *et al.*
653 2012), mutations in these genes could potentially lead to changes in sensitivity or
654 specificity of nerve activation, and combinations of mutations in different receptor
655 genes might be required for a complex modification of response to multiple
656 compounds differing between host plants. As ORs and GRs belong to large, fast
657 evolving gene families, and are the main peripheral discriminators, they are the best
658 *a priori* targets for involvement in host shifts. This assumption is supported by a
659 number of studies highlighting the involvement of chemoreceptors in differences
660 between host-associated races (McBride 2007; Smadja *et al.* 2012; McBride *et al.*
661 2014; Duvaux *et al.* 2015). In contrast OBPs and CSPs are smaller more conserved
662 families, more involved in presenting ligands to receptors. Although they have been
663 implicated in some host-shift cases (Matsuo *et al.* 2007; Dworkin & Jones 2009), we
664 find little evidence of their importance in pea aphid host-race formation. Duvaux *et al.*
665 (2015) and McBride (2007) both relate gain and loss of chemoreceptors to
666 between-race differences. Our finding of physical clusters of outlier genes (likely to
667 result from recent tandem duplications; Smadja *et al.* 2009) may suggest that
668 divergence after duplication is critical for the evolution of new response patterns
669 (figure 2).

670

671

672 *The genetic architecture of divergence between races*

673

674 Incorporating more races into our analysis has allowed us to identify a large number
675 of new chemosensory genes as potential targets of selection. It is clear that the same
676 set of genes is not necessarily involved in each adaptive host shift in the pea aphid;
677 different chemosensory genes were outliers on different axes of variation although
678 some axes separate multiple races. Identifying outlier genes in the more distinct
679 aphid races, such as those very divergent in *O. spinosa* and *C. scoparius*-associated
680 races, will be useful for follow-up work, as we cannot necessarily expect to examine
681 divergence at the same chemosensory outliers in all race comparisons. The
682 identification of multiple targets of selection relating to the same adaptive shifts
683 suggests a polygenic basis of local adaptation in the pea aphid, fitting with the
684 findings of Hawthorne and Via (2001), Caillaud and Via (2012) and Jaquiéry *et al.*
685 (2012) who all identified multiple QTLs relating to aphid plant choice. The overlap in
686 some cases between chemosensory genes identified on different axes of variation
687 (see supplementary file 6 and figure 2) also shows how the same chemosensory
688 genes can be involved in different adaptive host shifts, consistent with a
689 combinatorial model of chemoreceptor activation (Hallem & Carlson 2004; Carey &
690 Carlson 2011), and with the possibility that combinations or varying concentrations
691 of plant compounds may act to trigger host acceptance or rejection.

692

693 Smadja *et al.* (2012) found that outlier genes could be divided into those with mainly
694 non-synonymous substitutions and those with mainly synonymous site substitutions,
695 and took this to imply a role for regulatory changes in the loci with mainly
696 synonymous outliers, the high divergence at synonymous sites reflecting divergent
697 selection in closely linked regulatory regions. Consistent with this, we detected a
698 large number of putative promoter regions as outliers. Often an outlier putative
699 promoter was present upstream of a gene that was also identified as an outlier (e.g.
700 Gr4, Gr8 and Or18 in PC3 and Gr45 and Gr8 in PC4 of capture sequencing data). This
701 could be the result of hitchhiking in regions surrounding targets of selection, or it

702 could relate to evolution of gene expression in some receptors. Although
703 chemosensory genes as a class do not show more differential expression between
704 races than other genes, some chemosensory genes are significantly differentially
705 expressed between pea aphid races (Eyres *et al.* 2016); however, there is almost no
706 overlap between these differentially expressed genes and the putative promoters
707 identified here (ApisSNMP8 being the only exception).

708

709 The number of loci with extremely high loadings, equivalent to fixed differences,
710 between *La. pratensis*-associated aphids and the others was notably high, suggesting
711 the possibility of the accumulation of extensive neutral divergence between the *La.*
712 *pratensis*-associated race and the other races. In accordance with this, outlier SNPs
713 relating to *La. pratensis* contained a considerable number of control SNPs. Peccoud
714 *et al* (2009) suggested that the highly genetically differentiated *La. pratensis*-
715 associated race was nearing complete speciation, as no hybrid was detected with
716 sympatric races, and our results are consistent with the minimal gene flow estimated
717 between this more divergent race (Peccoud *et al.* 2009), in comparison to the higher
718 gene flow between more genetically-similar races, where loci experiencing barriers
719 to gene flow will stand out more clearly against a background of low differentiation
720 (Nosil *et al.* 2009; Butlin 2010). This pattern of increased neutral divergence in the
721 race with the lowest ongoing gene-flow supports the pea aphid host-race system as
722 a promising one for examining the genomic architecture of speciation with gene-flow
723 as races progress towards complete reproductive isolation.

724

725 *PCAdapt is a useful tool for analysing data with uncertain population assignment*
726 PCAdapt needs no prior information about assumed population membership of
727 samples. On the whole, aphids clearly clustered on the basis of the plant that they
728 were collected from. However, we detected multiple possible hybrids, as well as
729 migrant aphids that clustered with individuals sampled from a different host plant, in
730 our large SNP genotyping dataset. Because we were interested in identifying loci
731 relating to differences in host-plant preferences between races, using methods that
732 require *a priori* knowledge of population structure would have required us to
733 exclude or reclassify these individuals. Not doing this removed any artificial

734 population structuring, which could arise from removing intermediate or non-
735 conforming genotypes. In some cases (*P. sativum* and *T. pratense* in capture
736 sequencing data and *Lo. corniculatus* in GoldenGate SNP data), PCAdapt was able to
737 identify unexpected substructure within races, which are normally found to have
738 little substructure, regardless of spatial scale (Frantz *et al.* 2006; Peccoud *et al.* 2008;
739 Ferrari *et al.* 2012). The splitting of *P. sativum* and *T. pratense* individuals in the
740 capture sequencing data is particularly interesting, as it was not identified in the
741 analysis of the same data set (Duvaux *et al.* 2015) on the basis of 1777 SNPs and
742 random forest clustering. Instead, Duvaux *et al.* identified two clusters of the *M.*
743 *sativa*-associated individuals. For the most similar races, presumably with the most
744 recent origin and/or the highest gene flow, these findings may indicate that there is
745 some overlap between spatial and host-associated structure. This emphasizes the
746 value of avoiding prior classification, especially where this is based on a small
747 number of markers chosen for their ability to separate host races in a single region
748 (as with microsatellites often used in pea aphid studies (Jaquiéry *et al.* 2012)).

749

750 By looking at outliers relating to the principal components of genetic variation,
751 rather than looking for global or pairwise F_{ST} outliers, we get a more biologically
752 realistic insight into divisions between races, presumably looking at variation
753 reflecting historical population sub-division by host switching, and reflecting true
754 adaptive differences between relevant groups of races. This method also enabled us
755 to carry out far fewer comparisons – with six principal components of variation
756 rather than 28 pairwise comparisons between races – thus reducing the problems
757 associated with multiple testing.

758

759 *Conclusions*

760 The positive identification of outliers based on differences within and between
761 populations can be caused by many factors other than divergent selection.
762 Population size change (Teshima *et al.* 2006), population structure (Excoffier *et al.*
763 2009), and background selection (Stephan 2010) can all affect the detection of F_{ST}
764 outliers. Furthermore, once candidate loci have been identified, there are few cases
765 where their status has been confirmed in relation to phenotype or fitness impacts

766 (Jensen *et al.* 2016). The exceptions are in cases where, like in our analyses,
767 candidate loci were defined *a priori* (e.g. Colosimo *et al.* 2005; Hoekstra *et al.* 2006).
768 We have sound biological reasons for looking at these candidates, we have followed
769 them up in varied datasets and can confirm the outlier status of chemosensory
770 genes as a category as well as some specific Or and Gr genes. It is now important to
771 link these loci to behavioural differences between races, and to their assortative
772 mating, and to examine the genomic context of these potential targets of selection.

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Figure captions

Figure 1. PCAdapt scores for all pairwise combinations of principal components 1 to 6, after excluding *L. pratensis* associated aphids (K=6). Analysis based on 7232 SNPs from 104 aphid genotypes in 7 host-associated aphid races.

Figure 2. Loadings for each aphid genotype plotted for each principal component in turn. Outlier genes (Poisson test, $p < 0.05$) in boxes are associated with each principal component in the capture sequencing dataset (7232 SNPs, 104 aphid genotypes, 7 host-associated aphid races). Genes on the same scaffold (pea aphid genome V2.1) are bracketed together, genes with >2 outlier SNPs are in bold, and genes identified as outliers in Smadja *et al* (2012) are in red.

Figure 3. PCAdapt scores for all pairwise combinations of principal components 1 to 6, after excluding *L. pratensis* associated aphids (K=6). Analysis based on 179 GoldenGate SNPs from 373 aphid genotypes in 7 host-associated aphid races.

Figure 4. squared loadings for SNPs in each principal component of the GoldenGate SNP genotyping dataset plotted against squared loadings for the most strongly correlated principal component in the capture sequencing dataset (left to right, top to bottom: capture PC1 vs. SNP genotyping PC2, capture genotyping PC2 vs. SNP genotyping PC1, capture PC3 vs. SNP genotyping PC1, capture PC4 vs. SNP genotyping PC4, capture genotyping PC5 vs. SNP genotyping PC5, and maximum

squared loading capture genotyping vs. maximum squared loading SNP genotyping).

Black = control, pink = P450, green = chemosensory.

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Data accessibility: Capture sequencing reads are deposited in the EBI Sequence Read Archive (SRA) with project accession no. PRJEB6325. GoldenGate SNP genotypes are available in Supplementary File 5 tab 1, and the filtered set of capture sequencing SNPs used for PCAdapt analysis are available in Supplementary File 5 tab 2.

Author contributions: RKB, CS, JF and LD designed the study. JF, LD, DH and JCS collected and reared aphids for DNA extraction. IE, LD, KG and RT generated the capture sequencing and GoldenGate SNP genotype data. IE and RKB designed and performed the analyses. IE and RKB wrote the article. All authors commented on draft versions of the manuscript. Authors declare no conflict of interests.

Tables

Table 1: Outliers in each data set (Smadja *et al*, Capture Sequencing and GoldenGate SNP genotyping), for genes present in all data sets, two data sets and just one data set each. Smadja *et al* (2012) and Capture sequencing outliers with $p < 0.05$ Poisson probability of the observed or a greater number of SNP outliers given the number of SNPs in the gene and the overall proportion of outliers. Outliers from GoldenGate SNP genotyping are genes containing a SNP with a significant loading ($q < 0.05$) in PCAdapt.

		Analysed in:		
		All 3 data sets	2 data sets	1 data set
Outliers in:	All 3 data sets	Gr15, Or21, Or36		
	Smadja <i>et al</i> + capture	Gr45, Or17	Gr20, Gr47, Gr8, Or18, Or20	
	Smadja <i>et al</i> + SNP	Rad51C	-	
	Capture + SNP	Gr1, Gr2, Gr3, Gr33, Gr4, Gr6, Gr9	ApisSNMP4_ref	
	Smadja <i>et al</i> only	Or29	Gr39, Or11, Or13, Or14, Or15, Or51, Or56	Gr59, Or6, Or61, Or62, Or73
	Capture only	Gr17, Gr31, Gr65, Gr68, Or22, Or32, Or7	Gr10, Gr12, Gr19, Gr37, Gr42, Gr63, Gr66, OBP11, Or25, Or41, Or71, IR40a	Gr7, Gr74, OBP1, OBP4, ApisSNMP8_ref, IR8a
	SNP only	Gr21, Gr25, Gr26, Or16, Or26, Or3, Or47	Gr60, ApisSNMP3_ref	-
	Never an outlier	15	70	37