

## REVIEW

# On the scent of speciation: the chemosensory system and its role in premating isolation

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Chemosensory speciation is characterized by the evolution of barriers to genetic exchange that involve chemosensory systems and chemical signals. Here, we review some representative studies documenting chemosensory speciation in an attempt to evaluate the importance and the different aspects of the process in nature and to gain insights into the genetic basis and the evolutionary mechanisms of chemosensory trait divergence. Although most studies of chemosensory speciation concern sexual isolation mediated by pheromone divergence, especially in *Drosophila* and moth species, other chemically based behaviours (habitat choice, pollinator attraction) can also play an important role

in speciation and are likely to do so in a wide range of invertebrate and vertebrate species. Adaptive divergence of chemosensory traits in response to factors such as pollinators, hosts and conspecifics commonly drives the evolution of chemical prezygotic barriers. Although the genetic basis of chemosensory speciation remains largely unknown, genomic approaches to chemosensory gene families and to enzymes involved in biosynthetic pathways of signal compounds now provide new opportunities to dissect the genetic basis of these complex traits and of their divergence among taxa. *Heredity* (2009) **102**, 77–97; doi:10.1038/hdy.2008.55; published online 6 August 2008

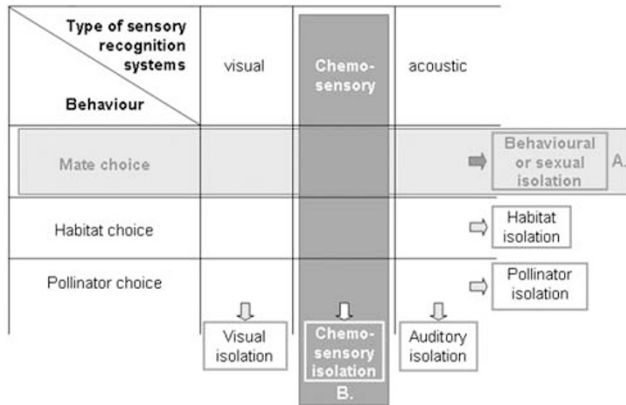
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## Introduction

If one accepts some version of the biological species concept, the central problem of speciation is the origin of isolating barriers that prevent actual or potential gene flow among populations (Coyne and Orr, 2004). Its solution requires identification of the traits involved in the reduction of gene flow among populations and understanding of the evolutionary forces that have acted on these traits.

When looking at the numerous studies conducted in the past few decades on speciation, one class of traits appears to play a fundamental role in premating isolation: the sensory systems and the stimuli they receive (which we will call 'signals' in this text). A sensory system consists of sensory receptors, neural pathways and parts of the brain that allow the integration of the information mediated by signals (visual, acoustic, tactile or chemical) present in the environment and possibly the initiation of a behavioural response to these signals (for example, discrimination, preference). In the literature, the role of sensory processes in speciation has been mostly illustrated by examples of 'behavioural isolation', which involves the divergence of a specific sensory system: the mate recognition system (for example, flycatchers: Saetre *et al.*, 1997; cichlids: Seehausen and Alphen, 1998; Hawaiian crickets: Shaw and Parsons, 2002; house mice: Smadja *et al.*, 2004). However, the role of sensory systems in speciation is not restricted

to behavioural isolation. Other modalities of premating isolation can involve the divergence of sensory traits (sensory systems and recognition signals): 'pollinator isolation' among angiosperm species due to the divergence of floral traits that promotes pollinator shifts (for example, floral colour divergence in the monkey flowers: Schemske and Bradshaw, 1999); 'habitat isolation' due to the divergence of host or habitat preferences (for example, apple maggot flies: Feder *et al.*, 1994). Although habitat and pollinator isolation both involve behavioural change, we will use the term behavioural isolation to refer more narrowly to traits directly affecting species recognition or mate choice. Modalities of 'sensory speciation' are thus diverse, according to the type of recognition event (among conspecifics as in mate recognition; with another species as in insect/host plant or plant/pollinator interactions) and the type of sensory system involved (visual, acoustic, chemosensory and so on; Figure 1). Compiling information across taxa and studies is necessary to assess the relative importance and the common features of these types of speciation processes. Most of the reviews that have been published so far have concerned behavioural isolation (for example, Butlin and Ritchie, 1994; Lambert and Spencer, 1995; Ritchie and Philips, 1998; Boake, 2002; Etges, 2002; Coyne and Orr, 2004; Ritchie, 2007), hence focusing on one type of behaviour (mate choice) but compiling examples across different types of sensory systems (Figure 1A). The present review addresses the role of sensory systems in speciation from a different perspective. We analyse the role of one type of sensory system in speciation, chemosensory systems, considering all types of behaviour that can be involved in premating isolation (chemosensory isolation; Figure 1B). To restrict our review, this article will not consider the chemical



**Figure 1** Modalities of 'sensory speciation'. This figure represents the different premating isolation modalities involving a divergence of sensory recognition systems. In columns, the different behaviours that can be involved in premating isolation are indicated; in rows, the different types of sensory systems potentially implicated in these different behaviours are indicated. 'Sensory isolation' modalities can be classified either by the premating barrier involved (for example, behavioural, habitat, pollinator isolation) or by the type of sensory system involved (for example, visual, chemosensory, auditory isolation). Light grey box (A) shows previous reviews that focused on behavioural isolation. Dark grey box (B) shows the present review that focuses on chemosensory isolation.

signalling involved in postmating prezygotic isolation (gamete/gamete or semen/reproductive-tract recognition; see Palumbi, this issue, for example) or chemical recognition among unicellular organisms or viruses, although we recognize the potential importance of these signals in speciation. Behavioural changes may often be the initial triggers for population divergence (Mayr, 1946; Butlin and Ritchie, 1994), and altered recognition, processing and response to chemical cues are expected to be involved in many behavioural changes. Consequently, understanding the role and relationship of chemosensory evolution to behaviour is likely to be fertile ground for understanding speciation. Chemosensory traits have been receiving considerable attention during the past decade but, so far, their role in speciation has not been reviewed specifically. We will use the term 'chemosensory traits' to include both signalling and sensory components of chemosensory systems and the terms 'chemosensory isolation' or 'chemosensory speciation' for premating reproductive isolation or speciation involving these traits.

Chemosensory systems, that is, olfactory and gustatory systems, are nowadays considered to be the most ubiquitous of all sensory systems (Ache and Young, 2005; Penn, 2006). Recent behavioural studies confirm this view. Although the critical importance of chemically based behaviours has been widely recognized in insects and rodents (reviewed in Wyatt, 2003), outstanding examples are now emerging in which odours regulate key life-history processes in various other taxa (for example, birds (Antarctic prions): Bonadonna and Nevitt, 2004; hermit crabs: Gherardi and Tiedemann, 2004; rotifers: Kotani *et al.*, 2001; fish (sticklebacks): McLennan, 2004). Understanding how these complex chemosensory traits evolve is, therefore, a major challenge for biologists. At the intraspecific level, many

studies have addressed the phenotypic and genetic characterization of odour-based behaviours (for example, moths: Löfstedt, 1993; *Drosophila*: Anholt and Mackay, 2001; mice: Brennan, 2004; Brennan and Keverne, 2004; for more examples, see Cardé and Minks, 1997). In comparison, relatively few studies have specifically investigated the evolution of chemically based behaviour at the suprapopulation level and its implications for speciation. Potentially, there are multiple layers of chemosensory change likely to be involved in behavioural shifts associated with premating isolation. First, the physiological changes can be due to alterations in peripheral signal input and/or processing of information by the central nervous system. Moreover, for example in insects, there can be changes affecting long range recognition or short range behaviours, changes affecting the gustatory or the olfactory systems. All these different possibilities interact ecologically and provide opportunities for evolutionary change leading to reproductive isolation. Here, we review some representative studies documenting chemically based speciation in an attempt to evaluate the importance and the modalities of chemosensory isolation in nature and gain insights into the genetic basis and the mechanisms of such divergence.

These evolutionary questions are addressed in the context of considerable recent progress in the molecular characterization of chemosensory systems and their stimuli. First identified in rats (Buck and Axel, 1991) and *Drosophila* (Clyne *et al.*, 1999), olfactory and gustatory receptor genes are now being characterized in a range of species as genome sequence data become available (Bargmann, 2006). As a result, the genomics of chemosensory systems can now help to unravel molecular features of chemoreception and to trace the mechanisms of chemical perception from molecules to behaviour (see for review in insects: Rutzler and Zwiebel, 2005; Benton, 2006; Hallem *et al.*, 2006; mammals: Dulac and Torello, 2003; and across phyla: Mombaerts, 1999; Firestein, 2001; Matsunami and Amrein, 2003; Ache and Young, 2005). By linking chemistry and physiology at one end with ecology and evolution at the other, 'chemogenomics' provides new opportunities to dissect the genetic basis of complex behaviour (Fitzpatrick *et al.*, 2005; Kurtovic *et al.*, 2007) and the functional genetic variation that underlies adaptation and reproductive isolation (Moyle, 2005; Clark, 2006; Noor and Feder, 2006; Storz and Hoekstra, 2007).

## Chemosensory traits in speciation

### Of moths and flies

When dealing with speciation based on chemicals, two groups of insects provide the classical examples: moths (Insecta: Lepidoptera) and drosophilid flies (Insecta: Diptera; all examples are described in Table 1).

In most moth species, mate finding involves long-distance signalling by female-emitted sex pheromones. The great diversity of pheromone structures used by moth species, and the extensive radiation seen in moths, led to consideration of the role of these pheromones in the speciation process (Linn and Roelofs, 1995; Greenfield, 2002). Closely related taxa often use the same major component, but the pheromones of related species can

**Table 1** Examples of chemosensory speciation

Species/pair of species	Chemoreception involved in	Gene flow	Some references <sup>a</sup>	Chemical characterization
<i>Insects</i>				
<i>Moths</i>				
Small ermine moths— <i>Yponomeuta</i> species	Behavioural isolation	No	1	Yes
Armyworms— <i>Spodoptera latifascia</i> and <i>S. descoinsi</i>	Behavioural isolation	No	2	Yes
Tobacco budworms— <i>Heliothis virescens</i> and <i>H. subflexa</i>	Behavioural isolation	No	3–8	Yes (3, 7)
Leafrollers— <i>Ctenopseustis obliquana</i> and <i>C. herana</i>	Behavioural isolation	No	9	Yes
Corn borers— <i>Ostrinia nubilalis</i> , <i>O. furnacalis</i> , <i>O. scapularis</i> , <i>O. zealis</i>	Behavioural isolation	No	10–12	Yes (10, 12)
Turnip moths— <i>Agrotis segetum</i> populations	Behavioural isolation	Yes	13–15	Yes
Nun moths— <i>Lymantria monacha</i>	Behavioural isolation	Yes	16	Yes
Common sheep moths— <i>Hemileuca eglanterina</i>	Behavioural isolation	Yes	17	Yes
Larch budmoths— <i>Zeiraphera diniana</i>	Behavioural and habitat isolation	Yes	18–19	Yes (18)
European corn borers— <i>Ostrinia nubilalis</i> races	Behavioural and habitat isolation	Yes	20–34	Yes (20, 24)
<i>Fruit flies, Drosophila</i>				
<i>D. melanogaster</i> subgroup ( <i>D. melanogaster</i> , <i>D. simulans</i> , <i>D. sechellia</i> , <i>D. mauritiana</i> )	Behavioural and habitat isolation	No	35–48	Yes (35, 38)
<i>D. pseudoobscura</i> and <i>D. persimilis</i>	Behavioural isolation	Yes	49–55	Yes (51)
<i>D. serrata</i> and <i>D. birchii</i>	Behavioural isolation	No	56–60	Yes (56, 57)
<i>D. santomea</i> and <i>D. yakuba</i>	Behavioural isolation	Yes	61–63	Yes (62)
<i>D. virilis</i> and <i>D. novamexicana</i>	Behavioural isolation	No	64	Yes
<i>D. elegans</i>	Behavioural isolation	Yes	65	Yes
<i>D. mojavensis</i>	Behavioural and habitat isolation	Yes	66–67	Yes
<i>D. melanogaster</i> races	Behavioural isolation	Yes	68–79	Yes (69)
<i>Others</i>				
Sulphur butterflies— <i>Colias eurytheme</i> and <i>C. philodice</i>	Behavioural isolation	Yes	80	Yes
Apple maggot flies— <i>Rhagoletis pomonella</i>	Habitat isolation	Yes	81–92	Yes (84, 85)
Bees— <i>Colletes cunicularius</i>	Behavioural isolation	Yes	93	Yes
Leaf beetles— <i>Chrysochus cobaltinus</i> and <i>C. auratus</i>	Behavioural isolation	Yes	94–96	Yes (96)
Bark beetles— <i>Ips pini</i> races	Behavioural isolation	Yes	97–99	Yes (98)
Pea aphids— <i>Acyrtosiphon pisum</i>	Habitat isolation	Yes	100–106	No
Aphids— <i>Aphis fabae fabae</i> and <i>A. f. mordwilkoii</i>	Behavioural isolation	Yes	107–108	Yes (108)
Meadow grasshoppers— <i>Chorthippus parallelus</i>	Behavioural isolation	Yes	109–110	Yes (110)
Walking-sticks— <i>Timema cristinae</i>	Behavioural isolation	Yes	111	Yes
<i>Mammals</i>				
Deer mice— <i>Peromyscus maniculatus</i> and <i>P. polionotus</i>	Behavioural isolation	No	112	No
Vlei rats— <i>Otomys irroratus</i>	Behavioural isolation	Yes	113	No
Gray leaf-eared mice— <i>Graomys griseoflavus</i> cytotypes	Behavioural isolation	No	114	No
Mole rats— <i>Spalax ehrenbergi</i> superspecies	Behavioural isolation	Yes	115–118	Yes (118)
House mice— <i>Mus musculus domesticus</i> and <i>M. m. musculus</i>	Behavioural isolation	Yes	119–126	No
<i>Squamates</i>				
Sea snakes— <i>Laticauda colubrina</i> and <i>L. frontalis</i>	Behavioural isolation	No	127	Yes
Red-sided garter snakes— <i>Thamnophis sirtalis parietalis</i>	Behavioural isolation	Yes	128	Yes
Iberian wall lizards— <i>Podarcis hispanica</i>	Behavioural isolation	Yes	129	Yes
Iberian wall lizards— <i>Podarcis hispanica</i> , <i>P. bocagei</i> and <i>P. carbonelli</i>	Behavioural isolation	No	130–132	No
<i>Fishes</i>				
Pupfishes— <i>Cyprinodon beltrani</i> , <i>C. labiosus</i> and <i>C. maya</i>	Behavioural isolation	Yes	133–134	No
Cichlids— <i>Pseudotropheus emmiltos</i> and <i>P. fainzilberi</i>	Behavioural isolation	No	135–136	No
Swordtail fishes— <i>Xiphophorus birchmanni</i> and <i>X. malinche</i>	Behavioural isolation	Yes	137	No
Swordtail fishes— <i>X. montezumae</i> , <i>X. nigrensis</i> and <i>X. cortezi</i>	Behavioural isolation	No	138–139	No
Three-spined sticklebacks— <i>Gasterosteus</i> spp	Behavioural isolation	Yes	140	No

Table 1 Continued

Species/pair of species	Chemoreception involved in	Gene flow	Some references <sup>a</sup>	Chemical characterization
<i>Annelids</i>				
Marine polychaetes— <i>Neanthes acuminata</i>	Behavioural isolation	Yes	141	No
<i>Plants</i>				
Orchids— <i>Ophrys fusca</i> and <i>O. bilunulata</i>	Pollinator isolation	No	142	Yes
Orchids— <i>Gymnadenia conopsea</i> and <i>G. odoratissima</i>	Pollinator isolation	Rare	143	Yes
<i>Silene dioica</i> and <i>S. latifolia</i>	Pollinator isolation	Yes	144	Yes

Gray shading indicates examples detailed in Tables 2, 3 and 4.

<sup>a</sup>Numbers correspond to references as follows: 1. Löfstedt *et al.*, 1991; 2. Monti *et al.*, 1997; 3. Pope *et al.*, 1982; 4. Groot *et al.*, 2004; 5. Groot *et al.*, 2005; 6. Sheck *et al.*, 2006; 7. Groot *et al.*, 2006; 8. Vickers, 2006; 9. Foster *et al.*, 1997; 10. Ishikawa *et al.*, 1999; 11. Roelofs *et al.*, 2002; 12. Tabata and Ishikawa, 2005; 13. Toth *et al.*, 1992; 14. LaForest *et al.*, 1997; 15. Wu *et al.*, 1999; 16. Gries *et al.*, 2001; 17. McElfresh and Millar, 2001; 18. Emelianov *et al.*, 2001; 19. Emelianov *et al.*, 2003; 20. Klun *et al.*, 1973; 21. Carde *et al.*, 1978; 22. Roelofs *et al.*, 1987; 23. Glover *et al.*, 1987; 24. Linn *et al.*, 1997; 25. Thomas *et al.*, 2003; 26. Dopman *et al.*, 2004; 27. Pelozuelo *et al.*, 2004; 28. Dopman *et al.*, 2005; 29. Malausa *et al.*, 2005; 30. Bethenod *et al.*, 2005; 31. Pelozuelo *et al.*, 2007; 32. Malausa *et al.*, 2007b; 33. Malausa *et al.*, 2007a; 34. Domingue *et al.*, 2007; 35. Jallon and David, 1987; 36. Rkha *et al.*, 1991; 37. Coyne, 1992; 38. Higa and Fuyama, 1993; 39. Coyne *et al.*, 1994; 40. Coyne and Oyama, 1995; 41. Jones, 2005; 42. Coyne, 1996b; 43. Coyne, 1996a; 44. Coyne and Charlesworth, 1997; 45. Civetta and Cantor, 2003; 46. Gleason *et al.*, 2005; 47. McBride, 2007; 48. Matsuo *et al.*, 2007; 49. Mayr, 1946; 50. Noor, 1995; 51. Noor and Coyne, 1996; 52. Noor *et al.*, 2001a; 53. Noor *et al.*, 2001b; 54. Ortiz-Barrientos *et al.*, 2004; 55. Ortiz-Barrientos and Noor, 2005; 56. Blows and Allan, 1998; 57. Higgie *et al.*, 2000; 58. Howard *et al.*, 2003; 59. Rundle *et al.*, 2005; 60. Higgie and Blows, 2007; 61. Coyne *et al.*, 2002; 62. Mas and Jallon, 2005; 63. Moehring *et al.*, 2006; 64. Doi *et al.*, 1996; 65. Ishii *et al.*, 2001; 66. Etges and Ahrens, 2001; 67. Newby and Etges, 1998; 68. Wu *et al.*, 1995; 69. Ferveur *et al.*, 1996; 70. Hollocher *et al.*, 1997a; 71. Hollocher *et al.*, 1997b; 72. Coyne *et al.*, 1999; 73. Dallerac *et al.*, 2000; 74. Takahashi *et al.*, 2001; 75. Ting *et al.*, 2001; 76. Fang *et al.*, 2002; 77. Greenberg *et al.*, 2003; 78. Ritchie and Noor, 2004; 79. Coyne and Elwyn, 2006; 80. Silberglied and Taylor, 1978; 81. Frey and Bush, 1990; 82. Feder *et al.*, 1994; 83. Feder *et al.*, 2003; 84. Nojima *et al.*, 2003a; 85. Nojima *et al.*, 2003b; 86. Linn *et al.*, 2003; 87. Linn *et al.*, 2005; 88. Forbes *et al.*, 2005; 89. Dambroski *et al.*, 2005; 90. Olsson *et al.*, 2006a; 91. Olsson *et al.*, 2006b; 92. Olsson *et al.*, 2006c; 93. Vereecken *et al.*, 2007; 94. Peterson *et al.*, 2005a; 95. Peterson *et al.*, 2005b; 96. Peterson *et al.*, 2007; 97. Teale *et al.*, 1994; 98. Seybold *et al.*, 1995; 99. Cognato *et al.*, 1999; 100. Pickett *et al.*, 1992; 101. Via, 1999; 102. Caillaud and Via, 2000; 103. Hawthorne and Via, 2001; 104. Via and Hawthorne, 2002; 105. Del Campo *et al.*, 2003; 106. Ferrari *et al.*, 2006; 107. Raymond *et al.*, 2001; 108. Park and Hardie, 2003; 109. Tregenza *et al.*, 2000; 110. Buckley *et al.*, 2003; 111. Nosil *et al.*, 2007; 112. Moore, 1965; 113. Pillay *et al.*, 1995; 114. Theiler and Blanco, 1996; 115. Nevo *et al.*, 1976; 116. Heth and Nevo, 1981; 117. Nevo *et al.*, 1987; 118. Menzies *et al.*, 1992; 119. Laukaitis *et al.*, 1997; 120. Kotenkova and Naidenko, 1999; 121. Talley *et al.*, 2001; 122. Smadja and Ganem, 2002; 123. Smadja *et al.*, 2004; 124. Smadja and Ganem, 2005; 125. Bimova *et al.*, 2005; 126. Smadja and Ganem, 2008; 127. Shine *et al.*, 2002; 128. Lemaster and Mason, 2003; 129. Martin and Lopez, 2006; 130. Cooper and Perez-Mellado, 2002; 131. Barbosa *et al.*, 2005; 132. Barbosa *et al.*, 2006; 133. Strecker and Kodric-Brown, 1999; 134. Kodric-Brown and Strecker, 2001; 135. Plenderleith *et al.*, 2005; 136. Blais, 2007; 137. Wong *et al.*, 2005; 138. McLennan and Ryan, 1997; 139. McLennan and Ryan, 1999; 140. Rafferty and Boughman, 2006; 141. Sutton *et al.*, 2005; 142. Schiestl and Ayasse, 2002; 143. Huber *et al.*, 2005; 144. Waelti *et al.*, 2008.

differ in the complement of minor components in the blend, or in the ratio of components produced (for example, specific ratios of geometric isomers usually of the major component). Numerous studies have analysed the production of specific pheromone blends by the females as well as the detection of the signal (electrophysiological antennal responses) and the induced behavioural responses in males (attraction to pheromone blends) among closely related moth species. In most cases, interspecific divergence in female pheromones correlates with a stronger attraction of males to conspecific signals. As examples, this has been shown in species of the genus *Yponomeuta* (Löfstedt *et al.*, 1991), *Spodoptera* (Monti *et al.*, 1997) and *Heliothis* (Groot *et al.*, 2006; see Table 1, with other examples). Assessing the variation of these systems within species is fundamental if one wants to understand how they have evolved during the process of speciation. Some studies have shown divergence in pheromone blends and responses among populations of a given species (Table 1; for example, *Agrotis segetum*: LaForest *et al.*, 1997; *Lymantria monacha*: Gries *et al.*, 2001; *Hemileuca eglanterina*: McElfresh and Millar, 2001; *Zeiraphera diniana*: Emelianov *et al.*, 2001). The best example is probably the case of two sympatric races of the European corn borer, *Ostrinia nubilalis*, in which pheromonal divergence is believed to cause partial behavioural isolation (Table 2).

In flies of the genus *Drosophila*, courtship and mating behaviours can involve visual and acoustic signals, and also olfactory and gustatory signals (reviewed, for example, in Markow and O'Grady, 2005). Chemical communication during courtship is thought to be mediated by the hydrocarbons found in the adult epicuticle (cuticular hydrocarbons; CHCs) that function as contact signals. CHCs can exhibit a remarkable degree of variability: they can differ in chain length, in the presence or absence of double bonds and in the position of the double bonds. Each individual produces a blend of CHCs and the characteristics of this blend can vary genetically as well as with age, sex, diet and geographic origin within a species (Ferveur, 2005). Some blends show diurnal rhythms (Kent *et al.*, 2007). Analyses of divergence in CHC blend among species suggest a role of CHCs in species recognition and speciation in *Drosophila* (for example, in *Drosophila mojavensis* group; Etges and Jackson, 2001). In the *Drosophila melanogaster* group, closely related species fall into two pairs with respect to CHCs: *D. simulans* and *D. mauritiana* are sexually monomorphic (with 7-tricosene as the predominant cuticular compound) whereas *D. melanogaster* and *D. sechellia* are sexually dimorphic (males: 7-T in *melanogaster*, 7-T + 6-T in *sechellia*; females: 7,11-heptacosadiene; Jallon and David, 1987). Asymmetric reproductive isolation occurs among these species: males of sexually dimorphic species will court females of all

**Table 2** The European corn borer, *Ostrinia nubilalis*

Like many moths, the European corn borer (ECB), *Ostrinia nubilalis* (Lepidoptera: Crambidae) uses a chemical communication system for long-distance mate attraction. In ECB two pheromone strains exist: one in which females produce and males respond to a 3:97 mixture of (E)- and (Z)-11-tetradecenyl acetate (Z strain) and another in which females produce and males respond to a 99:1 E/Z blend (E strain) (Klun *et al.*, 1973). Z-strain males fly to the 3:97 E/Z pheromone, but not to any other blends (Roelofs *et al.*, 1987). Most E-strain males respond to the 99:1 E/Z pheromone produced by E females but a substantial proportion fly to intermediate blends and an occasional male is attracted to the Z blend, leading to occasional hybridization when E males court and mate with Z females. Genetic studies assessing segregation patterns from F1, F2 and backcross progeny produced using Z- and E-strain parents indicate that pheromone production and male behavioural response are each determined by single major genes. Pheromone production exhibits autosomal inheritance, whereas male behavioural response is sex linked (Roelofs *et al.*, 1987). These loci, respectively called *Pher* and *Resp*, have been genetically mapped using AFLP and microsatellite markers (Dopman *et al.*, 2004, 2005). Differences in the pheromone blend produced by female ECB are likely due to changes in the specificity of the reactions in which D11-14-carbon-precursor acids are reduced and acetylated to produce the E and Z acetates, which are the pheromone components. Therefore, blends are likely generated by the differential specificity of alleles at a locus encoding a reductase (Roelofs *et al.*, 1987).

The European corn borer		
Signal divergence	Z strain	E strain
♀ long-range pheromone blend	3:97 mixture of E/Z- 11-14OAc	99:1 mixture of E/Z- 11-14OAc
♂ behavioural response	Preference for Z blend	Preference for E blend but cross attraction
↑ Link ? ↓		
Behavioural isolation	maize- Z race	hop-mugwort- E race
Matings	assortative	assortative

It has been suggested that sexual isolation could result from these stereotypic differences in male response to pheromone blend composition (Linn *et al.*, 1997). Behavioural isolation has been particularly studied between two sympatric host-plant races of the European corn borer. Populations of ECB feed on more than 200 weeds and cultivated plants and must have colonized maize (*Zea mays* L.) after its introduction into Europe, about 500 years ago. In France, ECB populations feeding on maize are genetically differentiated from sympatric populations feeding on mugwort (*Artemisia vulgaris* L.) and hop (*Humulus lupulus* L.). These two host races are reproductively isolated, as hybridization is very rare in the field (Malauza *et al.*, 2005). This cannot result solely from local mating on host plants, as ECBs usually mate in dense foxtail grass and other patches of herbaceous non host plants. At least two other factors decrease the frequency of hybridization: the allochronic emergence of the two host races (Thomas *et al.*, 2003) and the assortative mating of adult moths (prezygotic isolation; Bethenod *et al.*, 2005; Malauza *et al.*, 2005). Moreover, females of the two host races preferentially lay eggs on their natural host plant (Bethenod *et al.*, 2005). In France, the ratio of the Z and E isomers of the main pheromone component (11-tetradecenyl acetate) differs between the two races: the maize race pheromone blend contains a 97:3 Z/E ratio (Z blend) whereas the mugwort race blend contains an inverse ratio of 1:99 Z/E (E blend; Pelozuelo *et al.*, 2004). A straightforward explanation would be that their reproductive isolation is a mere consequence of 'assortative meeting' resulting from their different pheromones specifically attracting males towards same-race females at long range (Thomas *et al.*, 2003; Pelozuelo *et al.*, 2004). However, a recent publication showed that chemosensory isolation might not rely only on 'assortative meeting' via long-range pheromones but also, if not mainly, on a close-range mechanism that do not involve female long-range pheromone (Pelozuelo *et al.*, 2007). The nature of this mechanism remains unknown.

species, whereas monomorphic males will only court conspecific females. Experiments involving the transfer of CHCs from one species to the other demonstrated that sexual isolation in this group is largely caused by differences in female CHCs (Coyne *et al.*, 1994; Coyne, 1996a). Other pairs of closely related species show quantitative and/or qualitative differences in CHC blend and associated premating isolation (Table 1; for example, *D. virilis* and *D. novamexicana*: Doi *et al.*, 1996; *D. serrata* and *D. birchii*: Howard *et al.*, 2003; *D. santomea* and *D. yakuba*: Mas and Jallon, 2005 for preliminary results). Between *D. pseudoobscura* and *D. persimilis*, the fixed difference in CHC composition has not been shown to play a role in the sexual isolation of these species (Noor and Coyne, 1996), but the reinforced mating discrimination between the two taxa in sympatry seems to rely on olfaction (Ortiz-Barrientos *et al.*, 2004). Other studies demonstrate between-population variation within a

given species, as in *D. mojavensis* (Etges and Ahrens, 2001). Within *D. melanogaster*, divergence in CHC blend is observed between African and Caribbean populations (whose major CHC is 5,9-heptacosadiene) and 'cosmopolitan' populations from elsewhere in the world (whose major CHC is 7,11-HD). This divergence potentially underlies partial sexual isolation among populations of this species (Table 3).

These studies on moths and *Drosophila* underline how chemical signalling systems involving either long-range pheromones or CHCs often underlie behavioural isolation among closely related insect species. However, even in such systems that have received particular attention, it has to be noted that establishing the correspondence between assortative mating and the signals on which assortment is based is not straightforward. In *D. melanogaster*, uncertainty remains on the role of divergence in CHC blends (5,9-HD versus 7,11-HD) in

assortative mating between Z and M races (see Table 3). In *O. nubilalis*, the divergence in long-range pheromones has long been considered the key determinant of assortative mating among races. However, a recent study revealed that assortative mating does not rely solely on assortative meeting by long-range pheromones, but also involves short-range signals used when sexual partners are in contact (Pelozuelo *et al.*, 2007; see Table 2).

### Enlarging our view on chemosensory speciation

Beyond studies on moths and *Drosophila*, we report in Table 1 some representative studies that have examined patterns of speciation based on chemosensory traits in various species. We have selected these examples

on the basis of one main common feature: they all involve the differential recognition, and subsequently preference, for differentiated chemical signals, this preferential interaction between a given type of receiver and a corresponding type of chemical signal leading to premating isolation among populations or species. This compilation allows us to enlarge our view of chemosensory speciation and to evaluate the possible modalities of chemosensory isolation in nature.

**Modalities of chemosensory isolation:** The first information that emerges from these examples is that chemosensory speciation can involve different forms of premating reproductive isolation: mate choice, host

**Table 3** *Drosophila melanogaster*

In *Drosophila*, female cuticular hydrocarbons (CHCs) can act as mating pheromones (Ferveur, 2005). In much of Africa and the Caribbean, the major CHC on *Drosophila melanogaster* females is 5,9-heptacosadiene (5,9-HD). 'Cosmopolitan' females from elsewhere in the world, however, have much less 5,9-HD but large amounts of the isomer 7,11-HD.

In a first study investigating the genetic basis of such difference in CHC profiles, Ferveur *et al.* (1996) showed that it was controlled by loci on chromosome 3. Coyne *et al.* (1999) subsequently mapped a candidate gene to cytological region 87C-D on the third chromosome. Later molecular work confirmed the existence in this region of the *desaturase 2* gene (a  $\Delta 9$  fatty acid desaturase), and showed that the presence of the high 5,9-HD versus high 7,11-HD phenotype was completely correlated with the presence of 16 base-pair deletion in the putative *desat2* promoter region (Dallerac *et al.*, 2000; Takahashi *et al.*, 2001). Site-directed gene replacement later confirmed that the two alleles of *desat2* have a large effect on female hydrocarbon profile in the expected direction (Greenberg *et al.*, 2003). The allele with the deletion (called the 'D' or 'M' allele) produces the 'cosmopolitan' phenotype (high 7,11-HD), whereas the allele without the deletion (called the 'I' (insertion) or 'Z' (Zimbabwe) allele) produces the high 5,9-HD phenotype found in African and Caribbean populations (the allelic variation at this locus does not affect the CHC profile of males). It has been suggested that the distribution of these alleles reflects differential adaptation to climate: Z alleles are supposedly adapted to tropical conditions and M alleles to temperate ones (Greenberg *et al.*, 2003), although this result is still debated (Coyne and Elwyn, 2006; Greenberg *et al.*, 2006b).

A hypothesis suggested that the *desat2* polymorphism could cause sexual isolation between populations of *D. melanogaster*. Studies on sexual isolation in this *Drosophila* species have focused on the 'cosmopolitan' (M) and 'Zimbabwe' (Z) races of *D. melanogaster*. The former race occurs throughout the world, whereas the latter has been described from Zimbabwe, Zambia, and Botswana. In some parts of Africa, such as Zimbabwe, individuals of both races appear to be sympatric (Hollocher *et al.*, 1997b; Fang *et al.*, 2002; Takahashi and Ting, 2004). These races show marked but asymmetric sexual isolation: Zimbabwe-type females discriminate strongly against cosmopolitan males, whereas the reciprocal mating occurs readily (Wu *et al.*, 1995; Hollocher *et al.*, 1997a). This greater selectivity of Zimbabwe than that of cosmopolitan females, as well as whatever male traits are the objects of female discrimination, are based on several to many genes located on all three major chromosomes (Wu *et al.*, 1995; Hollocher *et al.*, 1997a; Ting *et al.*, 2001).

<i>Drosophila melanogaster</i>		
Signal divergence	Caribbean/ Africa	Rest_of_the_world
♀ Major Cuticular Hydrocarbon	5,9-HD	7,11-HD
<i>desaturase 2</i> alleles	<i>desat</i> <sup>Z</sup>	<i>desat</i> <sup>M</sup>
↕ Link ? ↕		
Behavioural isolation	Zimbabwe race (Z)	Cosmopolitan race (M)
♀ mate preference	assortative	random
♂ mate preference	random	random

Does this sexual isolation between the two *D. melanogaster* races rely on the divergence in CHC profiles? Although Fang *et al.* (2002) observed a positive correlation among African strains between the degree of female discrimination and the presence of the Z allele, evidence that *desat2* is involved in sexual isolation is not decisive. First, the correlation is limited as African and Caribbean populations both have the ancestral type hydrocarbon (5,9-HD), but Caribbean populations show M-type behaviour. Second, Coyne and Elwyn (2006) showed recently that sexual isolation between strains from nature takes the form of Z females rejecting the persistent courtship of M males while accepting Z males, excluding the implication of male choice. This is not the type of sexual isolation expected for *desat2*, which affects the CHC profiles of females but not of males. It seems implausible that a gene causing a difference in female (but not male) CHCs could simultaneously increase the selectivity of females as well as produce a male phenotype that is the object of this selection. However, we cannot exclude the possibility that *desat2* causes sexual isolation pleiotropically, through a phenotype unconnected with CHC profile. Whether the CHC polymorphism is truly a determinant of the Z/M behaviour differences or not remains to be tested.

choice or pollination. Therefore, we have classified these examples of chemosensory speciation according to the type of isolation in which chemoreception processes are involved (Table 1).

**Chemosensory behavioural isolation:** The examples previously described in moths and *Drosophila* species all referred to behavioural isolation, the mechanism by which isolation occurs through a reduction in attraction and/or probability of mating following contact among heterospecific individuals during the breeding season (Coyne and Orr, 2004). To date, chemosensory behavioural isolation has been studied much more widely than other modes of chemosensory isolation (Table 1). However, chemosensory traits can also be involved in other forms of premating isolation, all in the general category of ecological isolation.

**Chemosensory habitat isolation:** In animals, habitat isolation can limit reproductive encounters among heterospecific compared to conspecific individuals, premating isolation occurring as a byproduct of adaptation to different habitats (Coyne and Orr, 2004). Although many studies have documented habitat isolation by host shift, mainly in phytophagous insects (reviewed in Berlocher and Feder, 2002; Dres and Mallet, 2002), few of them have identified the cues underlying host preference. For those that have, it is clear that individuals recognize their host mainly through chemical stimuli. Examples of chemosensory habitat isolation are largely restricted to host-specific insects that recognize their host plant through chemicals emitted by the plant. In two sympatric host races of the pea aphid (*Acyrtosiphon pisum*) that are highly specialized on alfalfa and red clover (Via, 1999; Caillaud and Via, 2000), chemoreception seems to play a fundamental role in host acceptance and there is growing evidence that plant volatiles are important for host location (Park and Hardie, 2003). One of the most intensively studied cases is the habitat isolation among sympatric host races of the apple maggot fly, *Rhagoletis pomonella*, in which differential preference for host plant volatiles has been behaviourally and physiologically characterized (Table 4). Finally, some species that have been shown to use chemical cues in species recognition also recognize their host plant through chemosensory mechanisms (*D. sechellia* specialized on *Morinda* fruit, reviewed in McBride, 2007; *O. nubilalis* races on maize or mugwort/hop: Bethenod *et al.*, 2005; larch budmoth races on larch or cembran pine: Emelianov *et al.*, 2001; *D. mojavensis*: Newby and Etges, 1998). Another potential case concerns the homing behaviour of salmon. Generally, homing is precise and fidelity to the natal site results in reproductive isolation of spawning populations and in specialized adaptations of these populations for their natal habitat (Hendry, 2001). We know that homing is governed by olfactory recognition of home stream water (Dittman and Quinn, 1996). This information suggests that chemosensory habitat isolation could drive speciation in salmon, but this possibility has not been directly tested.

**Chemosensory pollinator isolation:** In flowering plants pollinated by specific pollinators, pollinator shifts can reduce the relative amount of heterospecific pollen reaching the stigma (Grant, 1994; Cozzolino and Widmer, 2005; Rieseberg and Willis, 2007). Floral odour

is an important trait used by many plants to attract pollinators. For instance, in sexually deceptive orchids, the pollination mechanism is based on mimicry of the sex pheromones released by the female insect and it has been speculated that the highly specialized nature of pollination by sexual deception has led to adaptive radiation accompanied by changes in floral scent (Ayasse *et al.*, 2000). Schiestl and Ayasse (2002) analysed the composition of floral odour and its olfactory detection by the pollinators in two sympatric sexually deceptive orchid species (*Ophrys fusca* and *Ophrys bilunulata*) and demonstrated that divergence in biologically active odour compounds in plants is responsible for pollinator isolation. Other examples of chemosensory pollinator isolation concern sympatric species of the genus *Gymnademina* and *Silene* (Table 1). We also know that pollinating fig wasps are attracted specifically to volatile chemicals emitted by receptive figs (for example, Grison-Pigé *et al.*, 2002) and that non-pollinating fig wasps locate their host through the recognition of fig chemicals (Proffitt *et al.*, 2007). However, investigations in these species-rich systems have not specifically addressed the role of chemical recognition in affecting reproductive isolation among either wasp species or fig species.

These examples show that the modalities of chemosensory speciation are diverse, the divergence affecting signals and behavioural responses being displayed either by individuals of the same species (mate recognition) or by different protagonists of a parasitic or mutualist interaction (for example, plant/insect). All these mechanisms have in common the evolution of chemosensory systems: olfaction is a candidate chemosensory process for sexual behaviour (long-range pheromones), host localization and pollinator attraction whereas gustation is expected to be involved in short-range recognition between sexual partners and for host acceptance.

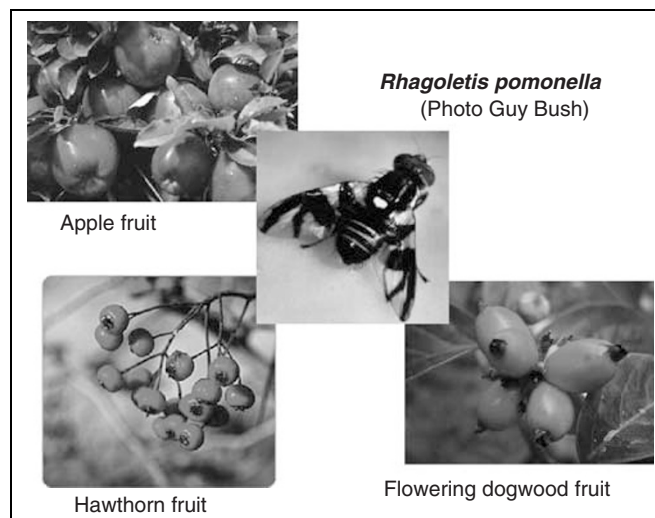
**Diversity of taxa influenced by chemosensory speciation:** The second inference from our review is the wide diversity of taxa involved. Although the data are heavily biased towards *Drosophila* and lepidopteran species, the phenomenon of speciation mediated by chemicals is documented in species belonging to various taxonomic groups, from mammals to plants (Table 1 for a complete list). Given that, as noted before, insects frequently employ chemical signals during courtship or host choice, it is not surprising that chemosensory speciation is documented in many insect species (for example, bees: Vereecken *et al.*, 2007; beetles: Peterson *et al.*, 2007; walking sticks: Nosil *et al.*, 2007; other references in Table 1), including some species primarily known for their use of acoustic cues in mate and species recognition (for example, meadow grasshoppers: Buckley *et al.*, 2003). Along these lines, divergence in CHCs has recently been shown among Hawaiian cricket species (Mullen *et al.*, 2007), although there is no evidence yet that CHCs serve as pheromones in these species or that this divergence could account for sexual isolation. There are likely to be many other examples of chemosensory speciation in insects: for instance in mosquitoes, in which a high degree of variation in chemical signals is known and used for taxonomy, speciation is largely unexplored (but see Caputo *et al.*,

**Table 4** *Rhagoletis pomonella*

The apple maggot fly, *Rhagoletis pomonella* Walsh (Diptera: Tephritidae) is a famous example of sympatric host races and a model for sympatric speciation by host-plant shifts. Two races, inhabiting hawthorn (*Crataegus* spp.) and apple (*Malus pumila*) trees occur sympatrically in the northeastern and midwestern United States. The recently derived apple-infesting population of *R. pomonella*, which originated by a shift from hawthorn in the mid-1800s, is considered as an example of host race formation in action, the hypothesized initial stage of sympatric speciation.

Reproductive isolation between host races is largely due to host choice because flies mate on their hosts with individuals that have made a similar choice and therefore differences in host preference translate directly into mate choice and premating reproductive isolation (Feder *et al.*, 1994). Therefore, understanding the role of host acceptance and fidelity in host race maintenance and speciation in *Rhagoletis* requires elucidating the mechanistic basis for differential host choice.

In the last few years, several studies have underlined the role of chemoreception as a key component of host acceptance in *Rhagoletis*. It has been shown that that volatiles emitted from the surface of ripening fruit are important long- and short-range chemosensory cues used to discriminate among potential host plants (Frey and Bush, 1990). More specifically, it was shown that, in both field and wind-tunnel experiments, apple flies are more attracted to volatile chemicals that are typical of apples than they are to those of hawthorn fruit (Linn *et al.*, 2003). Hawthorn flies and the closely related flowering dogwood fly, *Rhagoletis* sp. nov., do not show such a preference for apple volatiles, implying that the preference has recently evolved in apple flies. Moreover, apple, hawthorn and dogwood flies also tended to be antagonized by and avoid non natal fruit odours of alternative hosts (Forbes *et al.*, 2005; Linn *et al.*, 2005). Such preference for a unique mix of volatiles, and recognition of non-host volatiles, constitutes a basis for host fidelity and variation among individuals in a population provides a potential source for the host shifting process.



Discerning the chemical, physiological and genetic basis for fruit odour discrimination has therefore important ramifications for understanding how ecological specialization and population divergence can rapidly occur in the face of gene flow. In this regard, the relevant host volatiles attractive to each race of *R. pomonella* have been determined by gas chromatography/electroantennographic and flight-tunnel behavioural studies (Nojima *et al.*, 2003a,b). A first study investigating the genetics of host fruit odour discrimination in *Rhagoletis* reported that all F1 hybrid combinations of apple, hawthorn and dogwood flies were relatively insensitive to host fruit volatiles (Linn *et al.*, 2004), indicating that reduced olfactory host preference in *R. pomonella* hybrids could constitute an olfaction-based postzygotic barrier to gene flow as well. Later on, Dambroski *et al.* (2005) tested for a genetic basis for host fruit odour discrimination through an analysis of F2 and backcross hybrids constructed between apple-, hawthorn- and flowering dogwood-infesting *Rhagoletis* flies. Their results provide evidence that fruit odour discrimination differences among *R. pomonella* host races and sibling species have a genetic basis and that only a modest number of allelic differences at a few loci may underlie host fruit odour discrimination. Interestingly, recent studies also addressed the neurophysiologic basis of host choice. Through the use of single sensillum electrophysiology, some studies demonstrated that variation in olfactory receptor neuron threshold sensitivities and temporal firing patterns among the populations could influence host preference and contribute to sympatric host shifts (Olsson *et al.*, 2006a,b,c).

This progress in the characterization of the chemosensory basis of host preference in *Rhagoletis* will certainly provide in the future the opportunity to better understand how such levels of olfactory discrimination could be established within a mere 150 generations (as is the case for apple and hawthorn-origin flies), or indeed, exist in sympatry at all.

2007). We found only one example in other groups of invertebrates: in a marine polychaete (*Neanthes acuminata*), in which aggressive behaviour among populations, inducing premating isolation, relies on the olfactory detection of 'strangers' (Sutton *et al.*, 2005). There must surely be many others because it is known that arachnids, crustaceans and molluscs, among many others, use chemical cues in both habitat and mate choice. Many parasites must also use chemical cues in host location and so may be subject to chemosensory speciation through habitat isolation. At present, we have

no studies in these taxa that have directly addressed the role of chemical recognition in reproductive isolation.

In vertebrates, olfactory-based behavioural isolation has been demonstrated in some species of rodents (mainly in mole rats: Nevo *et al.*, 1976; house mice: Smadja *et al.*, 2004), squamates (sea snakes: Shine *et al.*, 2002; red-sided garter snakes: Lemaster and Mason, 2003; wall lizards: Martin and Lopez, 2006) and fishes (for example, cichlids: Plenderleith *et al.*, 2005; sticklebacks: Rafferty and Boughman, 2006; see Table 1). However, given the widespread interest in other aspects



of olfactory communication in rodents (Johnston, 2003; Brennan and Keverne, 2004), it is surprising how few studies have addressed the role of olfactory divergence in rodent speciation. Although some studies on rodents have demonstrated species-specific odours, very few have really tested the role of such species divergence in olfactory-based reproductive isolation (for example, Apps *et al.*, 1990). Conversely, although the most famous examples of speciation in fishes refer to visual cues (for example, three-spine sticklebacks: Rundle and Schluter, 1998; African cichlids: Seehausen and Alphen, 1998), olfaction also plays a significant role in speciation in some fish species. A recent example shows that adaptive divergence in MHC alleles influences odour-mediated mate choice and thus could have led to reproductive isolation among closely related African cichlid species (Blais, 2007).

Chemical characterization of the signals involved in divergence allows the qualitative and quantitative comparison among blends emitted by different populations or species. Characterization requires the collection and purification of the secreted chemicals and their identification by gas chromatography and sometimes mass spectrometry. Bioassays then allow the categorization of active compounds (Wyatt, 2003). Among examples of chemical reproductive isolation, chemical characterization has been achieved mainly in insects, reptiles and plants, in which methods are well established (for example, Gries *et al.*, 2001; Schiestl and Ayasse, 2002; Lemaster and Mason, 2003). In the other groups (rodents, fish, annelids), experiments during which individuals are presented with olfactory stimuli only (urinary stimuli for rodents; water perfumed with odours for aquatic animals) have demonstrated olfactory mate preferences (for example, Smadja *et al.*, 2004; Sutton *et al.*, 2005; Rafferty and Boughman, 2006). In the house mouse, the use of habituation techniques has allowed an indirect assessment of the extent of divergence in urinary signals within and between two subspecies (Smadja and Ganem, 2008). However, the specific odorant compounds involved in species or population recognition in these groups have not yet been characterized (but see Menzies *et al.*, 1992).

### Conclusion and perspectives

Studies on interspecific divergence among isolated taxa can reveal the potential role of chemical divergence in speciation. However, studies on taxa still exchanging genes in nature (Table 1) may be better models of changes contributing to the process of speciation because many differences among species may have accumulated after the speciation event and therefore be incidental to the process. Most of the existing studies have been performed in the laboratory and more studies in natural conditions are needed if one wants to understand how these chemicals act in a 'noisy' environment (for example, in the presence of chemicals released by other species). Speciation involving the evolution of chemosensory traits has been documented in a range of taxa, despite the taxonomic bias towards lepidopteran and dipteran insects. Given the number of species that seem to use chemical cues not only in mate choice (Wyatt, 2003; Johansson and Jones, 2007) but also in host choice and pollinator attraction, chemosensory speciation is

probably still largely unexplored. Chemical characterization of signals has been achieved in some cases, allowing the subsequent exploration of the mechanisms of signal divergence (see below). However, chemical characterization remains a challenge for the more complex olfactory signals (for example, urinary compounds).

### Evolution of chemosensory premating isolation

Given the role that chemosensory traits can play in speciation, one of the main challenges is to understand which evolutionary forces act on chemosensory traits to promote divergence among populations in diverse geographical contexts (allopatry, parapatry and sympatry). Although genetic drift alone can cause divergence, it is slow (Wu, 1985) and can only operate when extrinsic factors result in a low rate of gene flow. Therefore, selection is expected to play a role in the evolution of premating barriers but the form of selection is uncertain (ecological adaptation inadvertently or pleiotropically causing isolation, direct selection for isolation, sexual selection and so on) and interactions with drift may be important, for example following founder events or under Fisherian sexual selection.

Natural selection is expected to play a major role in the evolution of habitat isolation: adaptive differences in habitat use in allopatry or disruptive selection for resource use in the presence of gene flow can incidentally lead to reproductive isolation among populations (Coyne and Orr, 2004). Where local adaptation selects for habitat preferences (for example, Fry, 2003), it can have a more direct impact on the evolution of habitat isolation. The most widely accepted scenario for the evolution of pollinator isolation involves geographic separation of plant populations, divergence in floral traits then occurring as a result of disruptive selective pressures exerted by pollinators: for instance, plant populations experiencing completely different groups of pollinators or experiencing very different pollinator's preferences due to changes of the chemical environment (Coyne and Orr, 2004). Finally, the evolution of behavioural isolation has long been debated. The high specificity between the two components of the mate recognition system suggests that the signal/response channel should be highly canalized, with strong selection pressures against novel signals and preferences that would decrease the ability of individuals to obtain matings (Paterson, 1985; Lambert and Spencer, 1995; Greenfield, 2002). This view has led to the prediction that divergence in mating signals is more likely to occur through major shifts than through adaptive changes in small steps. However, it is now clear that variation in the mate recognition system commonly occurs within species and that selection acting on mate recognition systems can promote divergence of these traits among populations. Sexual selection is a force that acts specifically on mating systems and that can lead to accelerated diversification in mating preferences and signals within and among populations (reviewed in Ritchie, 2007), although the other types of selection can also be responsible for the evolution of behavioural barriers (for example, selection against hybridization, selection for species recognition, ecological selection and so on Coyne and Orr, 2004). Here, we

review studies addressing the evolution of divergence in chemosensory traits (phenotypic and genetic studies) and ask what these studies tell us about the mechanisms of chemical divergence and more generally about the evolution of premating barriers.

#### Inferences from phenotypic studies

**Indirect evidence from geographical patterns of divergence in natural populations:** By analysing patterns of geographical variation in a mating signal or preference among different populations of the same species, some studies have provided arguments in favour of the role of natural selection in the divergence of chemosensory traits. Gries *et al.* (2001) suggested that divergence in a mating signal in *L. monacha* populations from eastern Asia, relative to Central European populations, could have resulted from interspecific competition with the coseasonal *Lymantria fumida*. Similarly, the two pheromone types observed in *Hemileuca eglanterina* may have resulted from a reproductive character displacement (that is, greater divergence in a mating signal in a contact zone than in allopatry) in *H. eglanterina* populations in contact with a related species, *H. nuttali* (McElfresh and Millar, 2001). As these sympatric species do not hybridize in nature, it is unlikely that reinforcement (that is, selection against hybridization) has caused reproductive character displacement in these cases. Nevertheless, these examples suggest that communication interference among sympatric species that use similar premating signals can generate selective pressures on mating signals and lead to their divergence. Direct tests of this hypothesis are needed.

Other studies have analysed variation in chemical mating signals and preferences among populations of a single species that first diverged in allopatry and then met in secondary contact areas where they can still hybridize. These studies show patterns of reproductive character displacement affecting mating signals and/or mate preferences in various species: for example, between *D. pseudoobscura* and *D. persimilis* (Noor, 1995), between two subspecies of the house mouse (Smadja and Ganem, 2005, 2008), between the leaf-beetle species *Chrysochus cobaltinus* and *Chrysochus auratus* (Peterson *et al.*, 2005a) or among walking-stick populations (Nosil *et al.*, 2003). Evidence for reproductive character displacement, coupled with evidence for lower fitness of hybrids in the contact areas, argues in favour of reinforcement: selection against hybridization may have favoured divergence in mating signals and/or preference in contact zone populations to reduce the cost of production of unfit hybrid offspring.

These examples demonstrate, first, that variation in mate recognition system components can occur within a species' range. Second, they suggest a role for selective mechanisms (reinforcement, interspecific competition) in divergence among populations. Selection for efficient transmission of signals in different environments, known to play a role in the evolution of acoustic signals (for example, Ryan *et al.*, 1990), might also influence the divergence of chemical signals (Linn and Roelofs, 1995) although it has not been extensively addressed, as far as we know. However, one issue with analysing geographical variation is that it can only provide arguments in

favour of this role and not a direct demonstration. Many alternative scenarios can explain patterns such as reproductive character displacement and it is difficult to distinguish the alternatives with observational approaches (for a review, see Noor, 1999; Coyne and Orr, 2004).

**Direct evidence from experimental approaches:** In rare cases, experimental approaches have been used to evaluate the role of selection in the evolution of chemosensory speciation. Higgin *et al.* (2000) used experimental sympatry to demonstrate the role of natural selection in the generation of a field pattern of signal variation in *D. serrata*. Field populations of *D. serrata* display reproductive character displacement in CHCs when sympatric with *D. birchii*. By exposing field-sympatric and -allopatric populations of *D. serrata* to experimental sympatry with *D. birchii* for nine generations, they showed that CHCs of field-allopatric *D. serrata* populations evolved to resemble the field-sympatric populations, whereas field-sympatric *D. serrata* populations remained unchanged. Their experiment was one of the first to directly demonstrate that natural selection on mate recognition can result in a pattern of reproductive character displacement.

On the same species, Rundle *et al.* (2005) also used an experimental evolution approach—involving an ancestral laboratory environment and two novel resource environments—to evaluate the role of divergent selection in the evolution of female mating preferences. They found that CHCs evolved in response to the new environments and demonstrated that female mating preferences for these same CHCs also diverged among populations under different environmental treatments. This direct experimental manipulation showed that divergence in mating preferences for CHCs among *D. serrata* populations, expected to be an important source of premating isolation, can evolve, at least in part, as a byproduct of adaptation to the environment.

Finally, another study addressed experimentally the evolution of pheromone divergence between *Heliothis virescens* (Hv) and *Heliothis subflexa* (Hs). Although previous studies (Gries *et al.*, 2001; McElfresh and Millar, 2001) had only demonstrated the pattern of reproductive character displacement among moth species, Groot *et al.* (2006) conducted experiments to determine whether communication interference from males of these closely related species could exert strong enough directional selection to cause evolution of chemical mating signals in the face of stabilizing selection by conspecifics. Specifically, they first tested whether interspecific communication interference could be a directional selection force: they introduced quantitative trait locus (QTL) for low production of the acetate compound (typical of Hv female pheromone blend) into an Hs genetic background and measured, in field and cage experiments, the capacity of modified Hs females to attract and mate with Hs and Hv males. They showed that Hv males are more attracted to modified Hs females (with low acetates) than normal Hs females, suggesting that Hv males could have exerted directional selection on Hs females to produce relatively high amounts of acetates and so to avoid interference with the other species. They estimated that the directional selection exerted by Hv males on Hs females to produce relatively

high amounts of acetates can range from 0.135 to 0.231, demonstrating that interspecific selection may be intense enough to have counteracted intraspecific stabilizing selection and led to diversification of sexual signals.

Phenotypic studies have sometimes been successful in suggesting or more directly demonstrating the role of selective pressures in the evolution of divergence in chemosensory traits. However, all these studies refer to behavioural isolation. Moreover, evidence from patterns of variation in natural populations can only provide arguments in favour of a mechanism, whereas experimental approaches are limited to a few model species. This could explain why there are very few systems in which both chemical signals used in mate choice and the evolutionary forces influencing sexual isolation among species are known. In the following section, we will see how knowledge on the genetic basis of chemosensory traits can provide complementary information on the mechanisms of evolution of these traits, ideally up to direct tests for signatures of selection at the genomic level.

#### Inferences from genetic studies

Table 5 summarizes the genetic analyses that have been performed on premating barriers involving chemosensory traits, all of them involving insect species. Most of these genetic studies refer to behavioural isolation, some of them to habitat isolation and none to pollinator isolation. The genes underlying divergence in signals and preferences are likely to be distinct, even in the case of mate recognition systems (Butlin and Ritchie, 1989). As they have often been studied separately (which is unfortunate because they are expected to co-evolve), we present below, in separate sections, the information obtained so far on signals and preferences.

Most of the studies addressing the genetic basis of chemosensory speciation have used the tools of 'classical' genetics (Noor and Feder, 2006). This approach attempts to identify the number, distribution and type of genes that contribute to phenotypes that prevent gene flow among species, with a particular attention on the role of sex-linked genes and of 'major genes' (that is, single genes of large effect) in isolation. These methods have evolved to narrow down the search, ideally to the identification of specific genes involved in isolation (for an extensive review of these methods, see Ritchie and Philips, 1998; Coyne and Orr, 2004).

However, an alternative route to the identification of genes involved in chemosensory speciation is now available because of the growth of genomic approaches. Gene families involved in chemical signal production, chemoreception and processing have been described and they can be examined for loci that might contribute to divergence among populations or species. Clearly this candidate-gene approach can be particularly powerful to address the evolutionary genetics of chemosensory speciation when it is combined with classical genetic analysis (Joshi, 2005; Noor and Feder, 2006). Here we begin by reviewing the studies addressing genetics of divergence in chemical signals and in preferences and then describe the first fruits of candidate-gene approaches.

**Genetics of divergence in chemical signals:** Studies that have been published on the genetic basis of signal

divergence concern exclusively mating signals in insects (Table 5, light grey cells). One group of studies has demonstrated that single genes of large effect can be involved in causing signal divergence among closely related taxa. Changes at single loci underlie differences in pheromone blend among noctuid moth species (Monti *et al.*, 1997), races of European corn borers (Roelofs *et al.*, 1987; Dopman *et al.*, 2004) and *Ostrinia* species (Tabata and Ishikawa, 2005; Table 5). Moreover, an experiment on the cabbage looper (*Trichoplusia ni*) demonstrated the possible monogenic origin of pheromone divergence in the laboratory: in the course of a study involving collections of volatiles released by females, individuals releasing a new pheromone blend were discovered and one recessive autosomal gene has been shown to be involved in producing the mutant blend (reviewed in Haynes, 1997). When available, information on the biosynthetic pathway of these pheromone compounds helped to understand the mechanisms by which single genetic changes have led to divergence of signals among taxa. In *O. nubilalis*, the Z/E isomeric mixture is controlled by one reduction step at the end of the pathway, and the difference in the ratio of these isomers between the two races is due to a single change in the activity of the reductase controlling this final step (Roelofs *et al.*, 1987). In *T. ni*, the single mutation affects enzymes controlling chain shortening of precursors, this modification generating the three major differences observed between the mutant and normal pheromone blends (Linn *et al.*, 1997).

Several studies have also underlined the role of desaturase enzymes, which produce unsaturated fatty-acid precursors, in the emergence of new olfactory signals. The first striking example is the difference in CHC profiles between the two *D. melanogaster* pheromone races that has been shown to result from one mutation at a desaturase locus (*desat2*; Dallerac *et al.*, 2000; Takahashi *et al.*, 2001). A subsequent experiment, using site-directed gene replacement, later confirmed that the two alleles of *desat2* have a large effect on female CHC profile (Greenberg *et al.*, 2003; but see Table 3 for a complete discussion). In this case, divergence in olfactory signals results from the inactivation of a desaturase enzyme. Research on the desaturases in various moth species revealed that another way to produce a change in pheromone blend is by activation of a non-functional desaturase gene transcript present in the pheromone gland (Roelofs and Rooney, 2003): for instance, pheromonal differences between *O. nubilalis* and its Asian relative *Ostrinia furnacalis* result from the activation of an ancestral desaturase gene in *O. furnacalis*, found to be inactivated in all other *Ostrinia* species (Roelofs *et al.*, 2002). From these examples on desaturases, it is clear that the change occurred in a single step whereas large differences that map to a single locus, but have not been characterized at the molecular level, could, in principle, have accumulated by a series of substitutions of small effect.

Two conclusions can be drawn from these examples. (1) Signal divergence can occur through major shifts: single genetic changes can be responsible not only for simple signal differences, such as a ratio of isomer components (*O. nubilalis*, *D. melanogaster*), but also for alterations in multiple component blends (*Spodoptera*, *T. ni*) or qualitative differences (*O. nubilalis/furnacalis*;

**Table 5** Genetic analyses of chemically mediated premating isolation

Species/pair of species	Trait	Results	Some references <sup>a</sup>
<b>Behavioural isolation</b>			
Moths, <i>Spodoptera latifascia</i> and <i>S. descoinsi</i>	♀ Pheromone	1 autosomal gene	2
Moths, <i>Heliothis virescens</i> and <i>H. subflexa</i>	♀ Pheromone	Several QTL mainly on Chr4 and Chr22 Role of these QTL in the attraction of <i>Heliothis</i> ♀	6 4, 7
Moths, <i>Ctenopseustis obliquana</i> and <i>C. herana</i>	♀ Pheromone	> 1 gene	9
	♂ Response	1 single gene on ChrZ	
Corn borers, <i>Ostrinia</i>	♀ Pheromone	Activation of a <i>desaturase</i> pseudogene in <i>O. furnacalis</i>	11
<i>O. nubilalis/furnacalis</i>	♀ Pheromone	1 recessive autosomal gene	12
<i>O. scapularis/zealis</i>	♀ Pheromone	1 autosomal gene	22
<i>O. nubilalis</i> , Z and E pheromone races	♀ Pheromone	Chr12 (1 gene = <i>Pher</i> ). Candidate gene: <i>reductase</i>	28
	♂ Response	Response: 1 sex linked gene Pheromone-responding cells: 1 autosomal gene Response: ChrZ (1 gene = <i>Resp</i> )	22 28
<b>Fruit flies, <i>Drosophila melanogaster</i> subgroup</b>			
<i>D. simulans/sechellia</i>	♀ CHCs	Mapped to Chr3 7QTL for 7T: 2 on ChrX; 5 on Chr3; 3QTL for 7,11HD: Chr3; <i>desaturase 1</i> and 2 genes	39 46
	♂ CHCs	Mapped to ChrX; 2L+2R (≥2 genes); 3L+3R Chr3: 1 gene with large effect	43 45
	♀ Preference	≥2 genes	37
<i>D. simulans/melanogaster</i>	♀ CHCs	Mapped to 3R (> 1 gene); 3L (> 4 genes) = ≥5 genes	42
<i>D. mauritiana/sechellia</i>	♀ CHCs	Mapped to Chr3; ≥6 genes	44
<b>Fruit flies, <i>Drosophila pseudoobscura</i> and <i>D. persimilis</i></b>			
<i>D. pseudoobscura/D. persimilis</i>	♀ and ♂ CHCs	♀: Chr2; ♂: ChrX and Chr2	51
<i>D. pseudoobscura</i> allopatric/sympatric	♀ Reinforced discrimination	4 QTL: ChrX: <i>coy1</i> and 3; Chr4: <i>coy2</i> and 4 <i>Coy2</i> : one-allele assortative mating locus	54 55
<b>Fruit flies, <i>Drosophila santomea</i> and <i>D. yakuba</i></b>			
	♀ Discrimination	Likelihood of copulation: ≥1 on ChrX + ≥1 on autosome; copulation latency: ≥1 on ChrX	63
<b>Fruit flies, <i>Drosophila melanogaster</i> Afro-Caribbean and cosmopolitan strains</b>			
	♀ CHCs	Mapped to Chr3 1 single gene on 3R; candidate gene: <i>desaturase</i> Role of <i>desat2</i> gene in the biosynthesis of 5,9 HD 6-bp deletion in the 5' region of <i>desat2</i> gene	69 72 73 74
Z (Zimbabwe) and M (cosmopolitan) strains	♀ Preference	Mapped to three chromosomes: Chr3 > Chr2 > ChrX Chr3: ≥3 genes for Z females' preference	68, 70
	♀ CHCs	2 alleles of <i>desat2</i> gene	75 77
	♀ Preference	No link between <i>desat2</i> and sexual isolation	79
<b>Habitat isolation</b>			
<b>Fruit flies, <i>Drosophila melanogaster</i> subgroup</b>			
<i>D. sechellia/simulans</i>	<i>Sechellia</i> host preference for <i>Morinda</i> fruit	Recessive to <i>D. simulans</i> ' avoidance Chr2L: largest effect + Chr3R = ≥2 genes Lost of <i>Or</i> and <i>Gr</i> genes and positive selection and/ or relaxed constraint in <i>sechellia</i> (specialist)	36 38, 41 47
<i>D. sechellia</i> /other subgroup species	Host preference	Odorant binding protein genes ( <i>Obp57e</i> + <i>d</i> )	48
<b>Apple maggot flies, <i>Rhagoletis pomonella</i></b>			
Host races on apple, hawthorn, dogwood	Host discrimination	A few genes	89
<b>Pea aphids, <i>Acyrtosiphon pisum</i></b>			
Host races on alfalfa and clover	Host acceptance	7 QTL for alfalfa; 1 QTL for clover; QTLs for host plant acceptance might be linked to QTLs for host performance	103, 104

Abbreviations: CHC, cuticular hydrocarbon; Chr, chromosome; QTL, quantitative trait locus.

Light grey shading indicates signals, dark grey indicates preferences.

<sup>a</sup>Numbers correspond to the references cited in Table 1.

*T. ni*). (2) It seems that major shifts in pheromone composition are most likely to occur when the different compounds that compose the pheromone blend are products of a single biosynthetic pathway: although this system allows tight control over the production of the specific blend, a single mutation at one step of the biosynthetic pathway can lead to dramatic change in the pheromone composition (Linn *et al.*, 1997). Given this possibility for major shifts in chemical signals to be caused by single mutations, it is critical to understand how such mutations can spread when they are generally expected to reduce mate attraction. So far, the model of 'asymmetric tracking' offers a partial response: it predicts that a large mutational effect in female pheromone production can be tracked by male responses that are pre-adapted to a broader signal range (Phelan, 1992; Löfstedt, 1993). This prediction agrees with some observations: in *T. ni*, the male's behavioural response specificity in the mutant line has changed in response to the female's pheromone blend mutation (Haynes, 1997); in *O. nubilalis*, a few males are attracted to pheromones of *O. furnacalis* females, suggesting that some males were pre-adapted to respond to a novel pheromone (Roelofs *et al.*, 2002). However, this model does not explain how a new female pheromone can become fixed when only a few males respond to it. Neither is it clear why males respond to a much broader range of signals than is produced by females.

A second group of studies has shown that more than one genetic change (poly- or oligogenic basis) can account for signal divergence among taxa. Three sets of crosses have been performed between species pairs of the *D. melanogaster* subgroup involving a dimorphic species (either *D. melanogaster* or *D. sechellia*, where males predominantly produce monoenes, and only females have dienes) and a monomorphic species (either *D. simulans* or *D. mauritiana*, where both sexes produce 7-monoenes). In each cross, the variation of female-specific CHCs segregated with chromosome 3, and at least five non-overlapping genetic factors were detected on its left arm (3L; Coyne *et al.*, 1994; Coyne, 1996b; Coyne and Charlesworth, 1997). The genetic basis underlying the variation of the male-predominant monoene is different: the ratio between 7-T (in *D. simulans*) and 6-T (in *D. sechellia*; Cobb and Jallon, 1990) depends on factors dispersed on the five major chromosomal arms (Coyne, 1996a; Civetta and Cantor, 2003). The two species *D. pseudoobscura* and *D. persimilis* show a major difference in the ratio of methyl-hexacosane and (Z,Z)-5,9 heptacosadiene. This variation mostly depends on epistatic interactions between the X- and the second chromosomes, in both sexes (Noor and Coyne, 1996). Finally, quantitative variation of the principal CHC, (Z)-11-pentacosene, between a genetically marked strain of *D. virilis* and *D. novamexicana* segregated with two of the six chromosomes and one major genetic factor was mapped (Doi *et al.*, 1996; for a review of *Drosophila* work, see Ferveur, 2005; Gleason *et al.*, 2005). This polygenic basis is also found for female differences in long-range pheromone compounds between *H. virescens* and *H. subflexa* (Groot *et al.*, 2004; Sheck *et al.*, 2006).

A poly- or oligogenic basis can suggest that several small changes (gradual evolution) are responsible for signal divergence in these species. From studies on

moths, it appears that intrapopulation or geographical variation in pheromone blend is more likely in species that use combinations of components whose biosynthetic pathways are not linked and therefore are more difficult to regulate (for example, *A. segetum* populations; *Ctenopseustis obliquata/herana*: Linn and Roelofs, 1995). This variation within the signal channel can provide a source upon which either drift or selection (sexual or natural) can act and promote divergence among populations. Therefore, this genetic architecture is at least consistent with phenotypic observations suggesting that selection can act to promote gradual divergence in chemosensory traits that contribute to reproductive isolation. However, it has to be noted that all these examples refer to species that are already completely isolated, suggesting that these multiple genetic changes could have accumulated after the speciation event and thus might not be the cause of initial isolation. More generally, the distinction between a major gene and polygene is sometimes difficult to define. Several genetic changes can be responsible for the divergence of a trait (here signals) but a few QTLs of large effect, or identified genes, can explain much of the divergence, the 'polygenic' effect then becoming a major gene effect (for example, Gleason *et al.*, 2005).

Overall, the results of studies that have addressed the genetic basis of chemical signals suggest that divergence in a chemical mating signal can occur either by major shifts or more gradual changes. These different mechanisms may reflect constraints imposed by the biosynthetic pathways for producing and regulating more or less complex blends of components (Linn and Roelofs, 1995). A growing number of studies now suggest that desaturase genes play a central role in mating signal divergence in various insect species. In moths and flies, the evolution of these genes seems to have been influenced by gene duplication, gene loss and pseudogene formation, these processes being characteristic of the birth-and-death model of multigene family evolution (Roelofs and Rooney, 2003; Greenberg *et al.*, 2006a). As genome sequences become available in many more insect species, the development of genomic studies on desaturase genes is likely to be an important route to gain insights into the evolution of chemical signals and their role in insect speciation. In other groups, such as vertebrates, the genetic basis of pheromone divergence remains to be addressed, as well as the genetic basis of chemical signal divergence in plants.

**Genetics of divergence in chemosensory preferences:** Several studies have analysed the genetic basis of divergence in chemosensory preferences (involved in mate or habitat choice) that lead to premating isolation in insects (Table 5, dark grey cells). As with mating signals, the results indicate that the genetic basis of divergence in mating preference can be either simple or more complex: several genetic changes have been shown to be responsible for divergence in mate preference between *D. simulans*/*D. sechellia*, *D. melanogaster* Z and M races, *D. santomea*/*D. yakuba* and *D. virilis*/*D. novamexicana*, whereas difference in mate preference between the two *O. nubilalis* pheromone races and among *Ctenopseustis* species map to only one sex-linked locus (for *Drosophila* species, see Table 5; Takahashi and Ting, 2004; Markow and O'Grady, 2005). QTL studies addressing the genetic

basis of adaptation to different host plants in *D. sechellia*, apple maggot flies and pea aphids also showed that divergence in host preference relies on a few genetic changes (Table 5). Therefore, it seems that divergence in a complex chemosensory behavioural trait can have a relatively simple genetic basis, as was previously suggested by Ritchie and Philips (1998), but there are still not enough comparable cases to find a general pattern and so to make comparisons with other sensory systems. Beyond providing some information on the genetic architecture of behavioural divergence, some of these studies also helped to understand how this divergence may have evolved among taxa that are still exchanging genes in nature. A pair of studies investigated the genetic basis of variation within *D. pseudoobscura* for female mating discrimination that is enhanced by natural selection in areas of contact with *D. persimilis* (Ortiz-Barrientos *et al.*, 2004; Ortiz-Barrientos and Noor, 2005). QTLs responsible for reinforced mating discrimination mapped to candidate genes involved in olfaction. Intriguingly, the results indicated that one of these QTLs (called *Coy-2*) behaves as a one-allele assortative mating locus, that is, a locus where assortative mating is enhanced by increase in the same allele in both populations (Felsenstein, 1981), hence providing a plausible explanation for reinforcement of preferences despite gene flow between the two species. Similarly, the demonstration in the pea aphid that QTLs with antagonistic effects on performance on the two hosts are genetically linked to QTLs responsible for host acceptance suggests that this type of genetic architecture (close linkage or pleiotropy) has facilitated both the evolution of specialization and reproductive isolation among these sympatric populations (Hawthorne and Via, 2001; Via and Hawthorne, 2002).

Studies such as these provide important basic information on the number and location of genetic factors underlying changes in chemosensory behaviour, but the tools of classical genetics have rarely been successful in identifying specific genes at the molecular level. Therefore, it has not been possible to assess patterns of molecular evolution that may accompany evolution of host shifts or assortative mating.

**Candidate-gene approach to chemosensory speciation:** Since the identification of the chemoreceptor gene repertoire of *D. melanogaster* (Robertson *et al.*, 2003), several studies have exploited this new opportunity to decipher the molecular and physiological basis of signal integration (for example, Dekker *et al.*, 2006; Kurtovic *et al.*, 2007) and the evolutionary dynamics of chemoreceptor genes (Guo and Kim, 2007; Nozawa and Nei, 2007; Tunstall *et al.*, 2007). It is likely that the genes underlying chemosensory habitat and mate preferences that cause premating isolation are to be found among these chemoreceptor-encoding and chemoreceptor-related genes. We report, in Table 5, two studies that have addressed the genetic basis of host specialization in *D. sechellia*, relative to its generalist sibling species, using a gene-based approach based on publicly available chemosensory gene sequences. McBride (2007) examined the molecular evolution of *D. sechellia*'s entire suite of olfactory (*Or*) and gustatory (*Gr*) receptor genes, presumably including those involved in the specific adaptation of this species to *Morinda* fruit. Having

identified the entire repertoire of functional and non-functional *Or* and *Gr* genes in *D. sechellia* and *D. simulans*, she found that *D. sechellia* has lost *Or* and *Gr* genes nearly 10 times faster than its generalist sibling and that those receptors that remain functional in *D. sechellia* have fixed amino-acid replacement mutations at a consistently higher rate, relative to silent mutations, than their *D. simulans* orthologs. Thus, this study characterized the potential genetic signature of host specialization in an insect chemosensory system, the changes at *Or* and *Gr* loci being likely to reflect positive selection and/or relaxed constraint associated with the altered ecological niche of this fly. In the same year, Matsuo *et al.* (2007), by developing expression analysis and targeted mutagenesis experiments, identified two genes encoding odorant binding proteins, *Obp57d* and *Obp57e*, which are responsible for the behavioural differences between *D. sechellia* and its sibling species in their responses to the toxins contained in the ripe fruit of *Morinda citrifolia*.

These two pioneering studies underline the potential of the genomic approach: the identification of the genes underlying interspecific differences in chemosensory behaviours, among the whole repertoire of chemosensory genes, can help to gain insights into the evolutionary dynamics of these genes, providing evidence for selective constraint or response to positive selection within particular lineages for example, and thus can contribute to our understanding of the evolution of host adaptation, mate preferences and reproductive barriers. However, these approaches also have their limitations. It is dangerous to attribute differences between a single pair of species, such as *D. simulans* and *D. sechellia*, to a cause such as host specialization because there are many other potential causes amongst the differences in their ecology and demographic history. Gardiner *et al.* (2008) considered one alternative explanation in their analysis of the *Or* and *Gr* repertoires of the 12 *Drosophila* species for which genome sequences are now available. This group includes three host-specialist species (*D. sechellia*, *D. erecta* and *D. mojavensis*). An analysis of the proportion of pseudogenes (representing the rate of functional gene loss) showed that it is strongly correlated with genome size and differs slightly between island-endemic and continental species but does not differ between generalists and specialists. The general problem is that genomic data provide a great deal of information about a few species whereas comparative analysis across multiple taxa is needed to test hypotheses about association with environmental factors. This is true for inferences of selection as well as for genomic patterns such as rates of gene loss. It is also important to note that detection of the signature of a history of positive selection in gene sequences says nothing about the source of selection and this may be difficult to investigate because selection may not be operating in extant populations.

#### Future directions

Given the availability of genome sequences and the identification of chemosensory genes in a growing number of species (in insects: for example, mosquitoes, moths, honey bees, beetles; in other groups: for example, daphnia, rodents, fishes, platypus), gene-based approaches can now be developed in various biological models, providing new opportunities to address the

genetic basis of olfactory and gustatory preferences and of speciation. As chemoreceptors appear to evolve very rapidly (for example, Robertson and Wanner, 2006), it is not always possible to identify their coding sequences by sequencing them directly using other species' primers. However, at least in insects, chemoreceptors share common molecular features across species that allow the use of sequence and structural homology to identify them in new species genomes as genome sequencing is achieved (for example, Krieger *et al.*, 2004; Bohbot *et al.*, 2007). Thus, more non-model genomes are needed to address the molecular basis of chemosensory speciation and more generally of chemically based behaviours. Making the most of gene-based approaches requires combining them with classical genetic tools and, especially, with ecological and behavioural studies that can elucidate the selective factors acting on signals and preferences.

Moreover, the identification of genes underlying chemosensory preferences should be viewed as a source of candidate genes that can be investigated at the population level to assess variation within and among natural populations and their contribution to interspecific differences. Ideally, chemoreceptor genes should not be studied in isolation but in concert with candidate signal production loci such as the desaturase genes. In both cases, there are indications that gene family evolution, for example through gene duplication and loss, may be important as also the selection on individual loci. Do the different families, as well as individual loci within them, co-evolve during divergence and speciation? The availability of candidate loci also makes it possible to ask to what extent the evolution of species differences has involved changes in regulatory rather than structural genes.

A focus on receptor genes should not cause us to forget that divergence in chemosensory preference can rely on genetic changes in neurophysiological pathways. A series of studies on *R. pomonella* has addressed the physiological basis of divergence in olfactory preferences, showing that variability in peripheral sensitivity and temporal firing pattern could influence host preference and contribute to host fidelity and sympatric host shifts in the *Rhagoletis* complex (Olsson *et al.*, 2006a,c). Such physiological studies should be developed in other species and comparative analyses undertaken to assess the relative role of receptor and neurophysiological changes in the evolution and divergence of chemosensory preferences.

## General conclusion

The evolution of chemosensory systems and chemical signals plays an important role in the occurrence of premating isolating barriers in various taxa, even in those primarily known to use other types of recognition cues. Even though these cryptic and labile signals are often difficult to analyse and their divergence difficult to quantify, chemosensory speciation should be investigated more broadly. Given the growing availability of genomic data related to chemosensory traits, there are great new opportunities for the detailed dissection of the genetics of this specific speciation modality, up to the identification of genes or pathways that contribute to speciation. Especially in model species like *Drosophila*

and moths, full understanding of the mechanisms underlying chemosensory speciation will require ecological and behavioural studies of forces driving divergence in addition to these genomic studies. It will be important to develop comparative studies as well as try to fill some of the current big gaps in coverage, both taxonomic and for forms of isolation other than 'behavioural'. Moreover, our review focused on cognitive processes but chemosensory speciation is probably not restricted to this: speciation in microorganisms like fungi (Le Gac *et al.*, 2007; Giraud *et al.*, 2008), bacteria (Dettman *et al.*, 2007) or viruses (Duffy *et al.*, 2007) might involve chemical recognition mechanisms as well. Other sensory traits—visual, acoustic—are involved in speciation mechanisms. Comparative studies on the different cues involved in premating isolation could test the relative importance of chemically based mechanisms in speciation, but only when a wider range of species has been studied. Given the probable difference in the genetic and neurophysiological characteristics of these different sensory systems and signals, it would be interesting to go further in the comparison of these different 'sensory speciation' modalities and try to make specific predictions on speciation according to the system involved.

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