synthesize ascorbic acid in the liver[4,12]. Our results also confirm the results of Birney et al.[4] that the Echidna synthesizes ascorbic acid in its kidney.

A word about method: Birney et al.[4] assayed only the last enzyme in the pathway of ascorbic acid synthesis, L-gulonolactone oxidase[13]. This is the enzyme found to be missing in all species which show a dietary requirement for ascorbic acid. Therefore, when they found this enzyme present in 2 marsupial species of the family Macropodidae, they concluded that these animals synthesized ascorbic acid. To test for ascorbic acid synthesis we assayed the livers and kidneys of 2 species in the family Macropodidae, the tammar wallaby *(Macropus eugenii)* and the Eastern gray kangaroo *(Macropus giganteus)* and 1 species in the family Phalangeridae, the bushtailed possum *(Trichosurus vulpecula)* and could find no evidence of synthesis of ascorbic acid in these species. The lack of synthesis is not likely to be due to the low sensitivity of our assay methods. We obtained rates of ascorbic acid synthesis starting with sodium-D-glucuronate comparable to rates found by other workers[14]. The levels of ascorbic acid synthesis for the *Macropus* species found by Birney et al.[4] should be easily detected by our method. Since we could assay ascorbic acid added to the reaction mixture containing *Macropus* liver, we could assay the ascorbic acid if any had been synthesized. There are 2 possible explanations. The first is that the marsupials of the super-family Phalangeroidea, which includes both the families Macropodidae and Phalangeridae[14] but not the family Dasyuridae (marsupial mouse), have lost the ability to synthesize ascorbic acid because of a mutation in one of the other enzymes required rather than L-gulonolactone-oxidase. The second is that we obtained false negative results because of inhibition of synthesis of ascorbic acid in our crude extracts for some reason in the marsupials. The low value obtained for the marsupial mouse suggests the latter explanation, but the former should be excluded by further tests. The assay[5] for L-gulonolactone oxidase is the superior assay but can give false positive results since the animal may not be able to synthesize ascorbic acid because of a mutation in another enzyme. The assay using sodium-D-glucuronate tests whether the entire pathway is functional but can give false negative results. Thus, we suggest that both assays be carried out on each group of animals studied in the future.

Mean ascorbic acid synthesis (as μmoles ascorbate g · tissue^{-1} h^{-1}) in liver and kidney for 5 species of vertebrates

Species		Number	Kidney	Liver
Australian lungfish	*Neoceratodus forsteri*	2	Crossopterygii: Dipnoi 0.05	<0.02*
Frog	*Litoria raniformis*	4	Amphibia: Anura 0.54	<0.02*
Echidna	*Tachyglossus aculeatus*	1	Mammalia: Prototheria 1.09	<0.02*
Marsupial mouse	*Antechinus swainsoni*	4	Mammalia: Metatheria <0.02*	0.06
Laboratory rat	*Rattus norvegicus*	3	Mammalia: Eutheria <0.02*	0.98

* Minimum assayable amount.

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Response of the sawfly *Diprion similis* to chiral sex pheromones

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Summary. Males of *Diprion similis* respond to both the (+)-2R,3R,7R and (−)-2S,3S,7S enantiomers of its sex pheromone, erythro-3,7-dimethyl pentadecan-2-yl propionate. A mixture of the 2 enantiomers induces a response similar to that of the individual components.

Male diprionid sawflies have been shown to respond to esters of erythro-3,7-dimethylpentadecan-2-ol[2]. Each erythro ester exists in 4 chiral configurations, designated (+)-2R,3R,7R; (+)-2R,3R,7S; (−)-2S,3S,7S and (−)-2S,3S,7R. 2 *Neodiprion* species show a chiral specificity in their response to the pheromone. Both *Neodiprion lecontei* (Finch) and *N. pinetum* (Geoff.) respond to acetate isomers with a (−) erythro configuration[3,4]. A similar chiral specificity has been shown in sex pheromone responses of the Lepidoptera[5] and Coleoptera[6]. Our field trapping findings

Table 1. Field responses of male *Diprion similis* to the stereoisomers of 3,7-dimethyl-pentadecan-2-yl acetate and propionate*

	Experiment 1**		Experiment 2***	Experiment 3***	
(−)-2S,3S,7S propionate	24 (4–8)	b		14 (1–5)	b
(−)-2S,3S,7R propionate	0	c			
(+)-2R,3R,7R propionate	49 (7–12)	a		24 (2–8)	a
(+)-2R,3R,7S propionate	0	c			
(−)-2S,3S,7S acetate			0		
(−)-2S,3S,7R acetate			0		
(+)-2R,3R,7R acetate			0		
(+)-2R, 3R,7S acetate			0		
800 µg (−)-S,S,S propionate				18 (2–5)	a
800 µg (+)-R,R,R propionate					
Control (solvent)	0	c	0	0	c

* Total trap catches followed by the same letter in any experiment are not significantly different at $p < 0.05$; ANOVA followed by Newman Keul's test. Figures in brackets following total trap catch refer to range of individual trap catches. All treatments replicated 5 times. ** Duration 11 days. 800 µg of each compound presented. *** Duration 6 days. In experiment (2) 800 µg of each compound presented.

from a British population of the diprionid sawfly, *Diprion similis* Hartig, demonstrate that this species responds to both the (+)-2R,3R,7R propionate and its enantiomer.

Trapping was carried out at Kilvey Hill in Wales on a 27 ha plantation of Lodgepole Pine (*Pinus contorta* Dougl.) where there was a larval population of about 4000. Oecos delta traps (Oecos Monitoring Systems, Kimpton, Herts; 175 mm long × 100 mm wide), with removable sticky surfaces, were mounted on stakes 1 m above the ground. Test solutions of 99% pure erythro acetates and propionates, synthesized by Mori[7], were made in redistilled dichloromethane (Koch-Light) and applied to 7 × 20 mm rubber sleeve stoppers (West Pharmarubber Ltd).

In experiments 1 and 2 (table 1) the effectiveness of the 4 erythro acetates and propionates were investigated. No male sawflies were caught with any of the acetate isomers. Of the propionates, only the enantiomers (+)-2R,3R,7R and (−)-2S,3S,7S caught males; the (+)-R,R,R isomer being significantly more active than the (−)-S,S,S isomer. When the 2 active isomers were presented in a 1:1 mixture (table 1, experiment 3) no significant enhancement or suppression of trap catch was found. Cardé[8] has predicted that a genuine pheromone should exhibit increasing activity with increased dose. This was observed for *D. similis* with both the (+)-R,R,R and (−)-S,S,S isomers (table 2).

Trap catches and electroantennogram data obtained by Jewett[2,9] indicated that the sex pheromone of *D. similis* in North America was one of the erythro propionates. This is confirmed for the British race of this species, and our results further indicate that both enantiomers are active. This contrasts with the situation in *N. pinetum* where only the (−)-S,S,S propionate is fully active, and its enantiomer is completely inactive[4].

Table 2. Field responses of *Diprion similis* to a dilution series of the (+)-2R,3R,7R and (−)-2S,3S,7S stereoisomers of 3,7-dimethyl-pentadecan-2-yl propionate

Dilution	(+)-2R,3R,7R*		(−)-2R,3R,7R**	
0	0	c	0	c
8 µg	2 (0–1)	b	0	c
80 µg	7 (0–3)	b	4 (0–2)	c
800 µg	15 (1–5)	a	8 (1–2)	b
8 mg	17 (1–6)	a	11 (1–4)	a

5 replicates of each treatment. Total trap catches followed by the same symbol in each column are not significantly different at $p < 0.05$. Figures in brackets following total trap catch refer to range of individual trap catches. * Trap duration 6 days. ** Trap duration 5 days.

In general, insects respond to only 1 of the possible enantiomers of a chiral sex pheromone; the other enantiomer being ineffective[6] or even inhibitory[5]. Only 1 example of an insect responding to the enantiomer of its sex pheromone is known. In the boll weevil, *Anthonomus grandis*, females respond to both (+) and (−)-grandisol in equal numbers[10], although the natural pheromone is (+)-grandisol.

It is not known if female *D. similis* produce both active enantiomers. If only 1 is produced, and yet the males can respond to both, it considerably reduces the potential for the use of optical isomers of sex pheromones as isolating mechanisms in temporally sympatric species (e.g. *Diprion pini* and *D. similis*), as was suggested by Jewett[2,9]. Further studies are required to ascertain the enantiomeric composition of the pheromone produced by *D. similis*. If both enantiomers are emitted, a similar situation to that in *Gnathotrichus sulcatus* would apply. This beetle produces (+)- and (−)-sulcatol in a 65%:35% mixture, but responds optimally to a mixture of enantiomers over a broad range[11]. The ability of both the (+-2R,3R,7R and (−)-2S,3S,7S enantiomers to attract males may be explained by the existence of different receptors for each compound. The failure of the diastereomers (+)-2R,3R,7S and (−)-2S,3S,7R to attract males may be due to the absence of the appropriate receptors on the antennae. Kraemer[4] has postulated that in *Neodiprion* spp. only position 2 and 3 carbons are important for activity; it appears that in the related *Diprion similis* the position 7 also plays a crucial role.

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