taining 0.1% blue dextran (Pharmacia, Uppsala, Sweden). By this procedure one of the dilutions would ordinarily yield 30–300 colonies per plate. The plates were incubated at 37 °C for 5–7 days in a 95% N_2–5% CO_2 atmosphere and dextranase-producing colonies (DPC), identified by their haloes of degraded blue dextran[7], were counted along with total colonies for each plate. The percentage of DPC per quadrant was based on the overall count of DPC and total colonies for the 4 plates of each quadrant sample.

Results and discussion. All of the subjects showed DPC in the plaque samples from at least 1 of their quadrants and, for 28 subjects (14 from each group), these colonies were present in all of their quadrants. The majority of the DPC were *Actinomyces* species; most of the remaining DPC were strains of *Streptococcus mutans.* The data for the quadrants were evaluated statistically by a non-parametric (Kruskal-Wallis) test and by a weighted analysis of variance. The differences in mean DPC percentages among the quadrants within each group were determined, as well as the differences in mean DPC percentages between the corresponding quadrants of the 2 groups. None of the differences, either within groups or between groups, were significant at the 5% level.

In a separate comparison of the groups, each subject's total DPC and total colony count were used to calculate the overall DPC percentage for each subject. The table shows data for all subjects in order of increasing percentage ranges. Although the mean overall values for the groups were the same (3.15%), 13 of the 19 caries-free subjects showed percentages below 1.60 compared with only 5 of the 20 caries-active subjects. This trend is contrary to what might be expected if the DPC were significant anticaries factors for the caries-free subjects. One explanation for the results is that the caries-active recruits tend to be more infected by *S. mutans* than their caries-free counterparts[8], and thus probably have correspondingly higher levels of plaque glucans to promote the growth of dextranase-producing organisms. The prevalence of the organisms would then simply appear to reflect the availability of the glucan substrate.

It was concluded that this comparison of DPC levels between groups of caries-free and caries-active subjects did not provide sufficient evidence to support a caries-protective role for the DPC.

Percentage ranges of dextranase-producing colonies in plaque samples from caries-free and caries-active subjects

DPC percentage range	Number of subjects Caries-free (19)	Caries-active (20)
0.00–1.00	7	3
1.01–1.60	6	2
1.61–2.00	0	3
2.01–3.00	0	3
3.01–4.00	2	4
4.01–5.00	0	0
Above 5.00	4	5
Overall mean ± SD	3.15 ± 4.51	3.15 ± 2.29

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Evolutionary implications of ascorbic acid production in the Australian lungfish[1]

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Summary. It is shown that the Australian lungfish *(Neoceratodus forsteri)* synthesizes ascorbic acid in its kidney, suggesting that ascorbic acid synthesis started before the origin of the tetrapods.

The ability to synthesize ascorbic acid and the location of this ability in particular organs varies phylogenetically in vertebrates[3]. Amphibians, reptiles and egg-laying mammals (prototherians) produce ascorbic acid in their kidneys[3,4]. In the metatherian and eutherian mammals, ascorbic acid can be synthesized in the liver[3,4], but this ability has been lost in such divergent lines as those leading to the bats[5], anthropoid primates and guinea-pigs[3–5]. In birds, a similar evolutionary pattern has been found[6]. The bony fish are unable to synthesize ascorbic acid[7,8]. Thus Catterjee[3,8] has suggested that tetrapods developed the pathway for synthesizing ascorbic acid de novo. This suggestion cannot be true since the Australian lungfish synthesizes ascorbic acid in its kidney (table).

The Dipnoi, the sub-class to which the Australian lungfish belongs, the Rhipidista, the order from which the reptiles and amphibians arose, and the Achinopterygii, the class to which modern teleost fish belong, all seem to have arisen from a common ancestor some time before the middle Devonian (375 MYA)[9]. Since ascorbic acid synthesis is found in 2 of the 3 lines, it is likely that the common ancestor of all of these lines could synthesize ascorbic acid. Thus the lack of synthesis in the bony fish is likely to be a loss, as in the anthropoid primates, and the pattern of ascorbic acid synthesis in the primitive fish should be studied.

Ascorbic acid synthesis was determined using sodium-D-glucuronate as the substrate by the method of Chatterjee[10]. This method demands that all 3 of the enzymes required for the synthesis of ascorbic acid[11] be active. The table shows that a marsupial mouse synthesizes ascorbic acid in its liver. This agrees with the finding that other marsupials

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