

Size and growth modification in clownfish

Sex change is not the only way these fish achieve dominance — they grow into the role.

Conflicts of interest are part and parcel of living in a social group, although these can reduce the fitness of individual members. Here I show that clownfish (*Amphiprion percula*) adjust their size and growth rate according to their position in the group hierarchy, maintaining a well-defined size difference with respect to individuals above them in social rank. This strategy to prevent conflict is a surprising departure from the more usual ploy used by many animals of modifying their behaviour within the group^{1,2}.

In Madang Lagoon, Papua New Guinea, groups of *A. percula* inhabit sea anemones, which protect them from predators (Fig. 1)^{3,4}. Each group is composed of a breeding pair and between zero and four non-breeders^{5,6}. Within each group, there is a size-based dominance hierarchy: the female is largest (rank 1), the male is second largest (rank 2), and the non-breeders get progressively smaller as the hierarchy is descended (ranks 3–6; ref. 5). If the female of a group dies, the male changes sex and becomes the breeding female, while the largest non-breeder becomes the breeding male⁵.

Subordinates benefit from settling in an anemone and queuing for breeding positions^{7,8}, their chances of success being determined by their rank within the group^{8,9}. Dominants, however, gain no benefits from subordinates, which are potential challengers for their position (P.B., unpublished results). This asymmetry generates evolutionary conflict over subordinate group membership, and dominants occasionally



Figure 1 Clownfish modify their size, rather than their behaviour, to ensure harmony in social groups.

evict¹⁰ or kill³ subordinates that are of similar size to themselves. Conflict would be avoided, however, if a size difference could be maintained between individuals adjacent in rank that meant that a subordinate would never present a threat to its immediate dominant.

A multivariate analysis reveals that there are well-defined size differences between individuals adjacent in rank (Fig. 2a): the standard length of subordinates (ranks 2–6) is positively related to their rank, the standard length of rank 1, and the number of individuals in the group (SAS mixed

procedure: rank, $F_{4,137} = 325.71$, $P < 0.0001$; standard length rank 1, $F_{1,137} = 105.37$, $P < 0.0001$; number of individuals, $F_{4,137} = 14.13$, $P < 0.0001$; $R^2 = 0.82$), whereas anemone diameter, depth and reef had no significant effect.

These well-defined size differences are maintained by the regulation of growth in response to social context, as illustrated by an experiment in which the rank-2 fish is removed from the group (Fig. 2b). In this experiment, the growth of rank-3 fish is positively related to the removal of the fish of rank 2 and the growth of the fish of rank 1, and negatively related to its own residual standard length (the difference between observed and expected measures) (SAS mixed procedure: removal, $F_{1,48} = 255.82$, $P < 0.0001$; growth of rank 1, $F_{1,48} = 6.22$, $P = 0.0161$; residual standard length, $F_{1,48} = 76.12$, $P < 0.0001$; $R^2 = 0.89$), whereas its initial standard length, anemone diameter, depth and reef had no significant effect.

The removal of the rank-2 fish caused the rank-3 fish to ascend in rank. Subordinates that ascended in rank grew more than individuals that did not ascend in rank. The residual standard length indicates how much smaller (negative residuals) or larger (positive residuals) the subordinate's observed standard length was compared with its standard length predicted by its social context (Fig. 2a). When the initial standard length of a subordinate was less than predicted, it grew more than an individual whose initial standard length was greater than predicted.

The coefficients of the size analysis

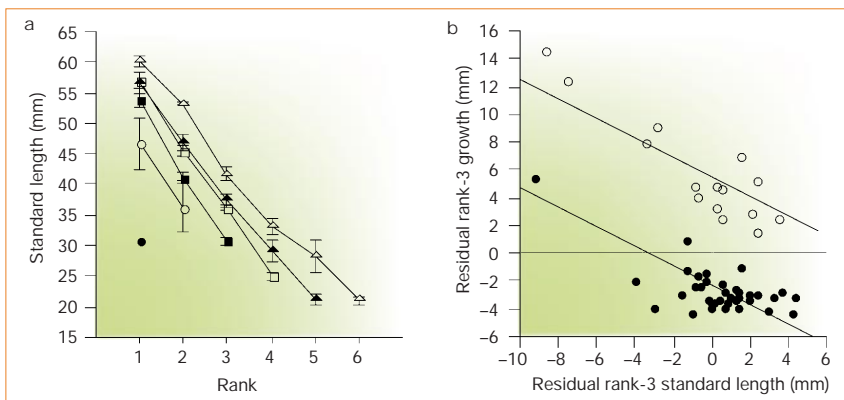


Figure 2 Size and growth of clownfish (*Amphiprion percula*) in relation to rank. **a**, Standard length (SL) of subordinates (ranks 2–6) is related to their rank, the SL of rank 1, and the number of individuals in the group ($n = 237$ individuals in 96 groups). Symbols represent the mean SL for each rank and group size (different symbols correspond to different group sizes of one to six fish); bars represent standard error. SL was measured in January 1997. **b**, Growth of fish of rank 3 was related to the removal of the fish of rank 2, the growth of the rank-1 fish, and their own 'residual' SL (control, filled circles, $n = 36$; experimental, open circles, $n = 16$): residual rank-3 SLs are the residuals of the model demonstrated in **a**. Residual rank-3 growth values are the residuals of a reduced model showing that the growth of rank 3 is related to the growth of rank 1. Fish of rank 2 were removed in February 1997; growth was calculated as the change in SL between January 1997 and December 1997. Individuals were identified by the natural variation in their colour markings.

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indicate that the average difference in standard length between fish of ranks 2 and 3 was 10.1 mm (Fig. 2a). The coefficients of the growth analysis indicate that individuals of rank 3 that were experimentally elevated to rank 2 had the same growth rate as control individuals (rank 3) when their standard length was 10.8 mm greater than that of controls (Fig. 2b). The similarity of these two estimates shows that the size difference that is found between individuals adjacent in rank would be restored after the disappearance of intermediate individuals.

These findings show that there are well-defined size differences between individuals adjacent in rank within groups of *A. percula*, and that these are maintained by the precise regulation of subordinate growth. The maintenance of size differences may resolve evolutionary conflict over group membership, because subordinates do not become a threat to their dominants. The results indicate that the growth rate

and the size adopted by any group-living organism could be a strategic response to its social environment.

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Hydrogen bonding

Single enantiomers from a chiral-alcohol catalyst

Hydrogen bonding acts as a ubiquitous glue to sustain the intricate architecture and functionality of proteins, nucleic acids and many supramolecular assemblies^{1,2}, but this weak interaction is seldom used as a force for promoting chemical reactions^{3–5}. Here we show that a simple chiral alcohol uses hydrogen bonding to catalyse an important family of cycloaddition reactions of a diene with various aldehydes — moreover, this reaction is highly enantioselective, generating only one of the mirror-image forms of each dihydropyran product. This type of catalysis mimics the action of enzymes and antibodies, and is unlike traditional, metal-based catalysts used in organic chemistry⁶.

Important biological molecules such as DNA and proteins, and therefore many pharmaceutical drugs, are chiral — that is,

they are not superimposable on their mirror image (the pair of asymmetric molecules are known as enantiomers), so any chemical reaction that can selectively synthesize one enantiomeric form of a chiral compound is potentially very useful.

Candidate catalysts for such reactions are generally based on Lewis-acid metals⁶. On the basis of our discovery that hetero-Diels–Alder (HDA) reactions⁷ between unactivated ketones and 1-amino-3-siloxy diene (compound **1**) are accelerated in protic solvents, we investigated the use of chiral alcohols for the asymmetric catalysis of these cycloadditions⁸. Of the possible alcohols that could be used for the HDA reaction between diene **1** and benzaldehyde, the TADDOL ($\alpha,\alpha,\alpha',\alpha'$ -tetraaryl-1,3-dioxolan-4,5-dimethanol) class of chiral alcohols, which are known to complex with carbonyl groups, was considered promising⁹.

Figure 1 shows the scheme of the HDA reaction in the presence of this chiral alcohol. A solution of 0.1 mmol (*R,R*)-1-naphthyl TADDOL (**2**) and benzaldehyde

(1.0 mmol) in toluene at -78°C was treated with diene **1** (0.5 mmol) and stirred, causing a smooth HDA reaction to take place. Analysis of the reaction mixture by ¹H NMR indicated that the cycloadduct had been formed as a single diastereomer (**3**, where R is C₆H₅), tentatively assigned as *endo*. On treatment with acetyl chloride (1.0 mmol), the cycloadduct was converted to dihydropyrene (**4**, 70% overall yield).

Analysis of the product by high-performance liquid chromatography revealed that the *S*-enantiomer had been produced preferentially over the *R*-enantiomer (>99:1). The reaction is considerably accelerated by TADDOL (**2**): in its absence, there was no reaction under otherwise identical conditions. Moreover, the monomethyl and dimethylether derivatives of **2** were poor catalysts, indicating that the hydrogen-bonding capability of **2** is crucial for the catalytic function.

This metal-free asymmetric catalysis, which does not involve a covalent connection between the catalyst and the reactant¹⁰, can be used for cycloadditions between **1** and a range of aldehydes with different R-groups (Fig. 1). Aromatic aldehydes were particularly effective as dienophiles in these HDA reactions. The resultant dihydropyrene products (see **4a–f** in supplementary information) were also consistently obtained with high enantiomer ratios. Aldehydes with aliphatic (**4g**) and α,β -unsaturated (**4h**) R-groups could be used successfully in these reactions.

Our results show that hydrogen bonding by a simple chiral alcohol to a carbonyl group can accomplish what has previously been considered to be in the domain of enzymes, catalytic antibodies and chiral metal-based Lewis acids. These studies indicate the broad potential for hydrogen-bond catalysis in asymmetric synthesis.

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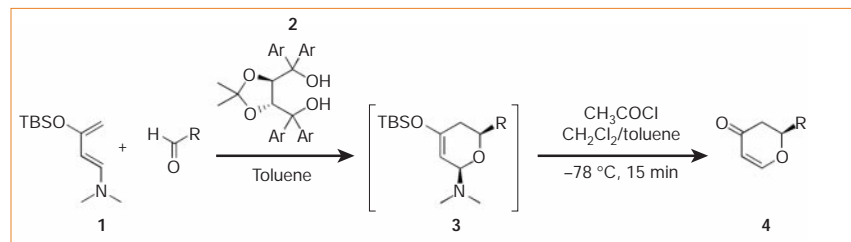


Figure 1 Asymmetric hetero-Diels–Alder reaction of diene **1** with aldehydes to produce dihydropyrenes (**4**). The reaction is catalysed by hydrogen bonding and yields specific enantiomeric molecules (see supplementary information for eight examples of products — molecules **4a–h** — bearing different R-groups). TBS, *tert*-butyldimethylsilyl derivative; Ar, 1-naphthyl group. The experimental procedure involved adding diene (**1**, 0.5 mmol) to a solution of TADDOL (**2**, 0.1 mmol) and the aldehyde (1.0 mmol) in toluene (0.5 ml) cooled to -40°C (-78°C for **4a**, **4c** and **4f**). The mixture was stirred for 24 h at -40°C (48 h for **4a**, **4c** and **4f**) and then diluted with CH₂Cl₂ (2.0 ml). Acetyl chloride (1.0 mmol) was added dropwise at -78°C , and the mixture was stirred for a further 15 min and then separated by chromatography on silica gel (yields 52–97%; enantiomer ratio 96:4→99:1).

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