Fig. S1. Intraspecific variability topographic distribution of ganglion cells and cone photoreceptors (single, double and total cones) in Rhinecanthus aculeatus. The black lines represent iso-density contours and values are expressed in densities \( \times 10^3 \) cells/mm\(^2\). The black arrow indicates the orientation of the retinas. T = temporal, V = ventral. Scale bars: 1 mm.
Behavioural measurements of achromatic and chromatic acuity

Fig. S2. i) Spectral reflectance of laminated stimuli used in each treatment labelled by color: pink, purple, green and yellow; irradiance of sidewelling lights measured in the testing tanks shown as dashed line; ii) Spectral sensitivities of the three cones in triggerfish, *Rhinecanthus aculeatus*, with a single cone sensitivity of $\lambda_{\text{max}} = 413$ nm, and double cone sensitivities of $\lambda_{\text{max}} = 480$ nm and $\lambda = 528$ nm multiplied by yellow cornea (Cheney et al., 2013); iii) Experimental tank setup; iv) An example of the achromatic (black and white) square-wave gratings: a) 1 cycle per degree; b) 5 cycles per degree.
Table S1. Primers used for probe template synthesis of fluorescence in-situ hybridisation. T7 (forward primer) and T3 (reverse primer) RNA polymerase promoter sequences are shown in bold.

| Target gene | Primer | Sequence |
|-------------|--------|----------|
| RH2A        | RH2A_F1, RH2A_R1 | 5'-TAATACGACTCACTATAGGGATGACAAGCTCTGGCTTG-3'  
              |        | 5'-AATTAACCCTCACTAAAGGGTTTTTGCAAAGAAGGCGAC-3'  |
| RH2C-1 & RH2C-2 | RH2C_F1, RH2C_R1 | 5'-TAATACGACTCACTATAGGGCCATCAACTTCCTGACGCTA-3'  
              |        | 5'-AATTAACCCTCACTAAAGGGAGCCAAACCATCAGGACA-3'  |
| SWS2B       | SWS2B_F1, SWS2B_R1 | 5'-TAATACGACTCACTATAGGGAGAGCCCAGATGACTCTCT-3'  
              |        | 5'-AATTAACCCTCACTAAAGGGACGGACTGACTGATGAGGA-3'  |
**Table S2.** Summary of transcriptomes, opsin mapping and opsin gene expression of *Rhinecanthus aculeatus.*

| Origin | ID        | # filtered transcriptome reads | RH1 # reads | Single cones (SC) | RH2A # reads | RH2C-1 # reads | RH2C-1 # reads | LWS # reads | Rod vs Cone | SC | DC |
|--------|-----------|--------------------------------|-------------|-------------------|-------------|----------------|----------------|-------------|-------------|----|----|
|        |           |                                |             |                   |             |                |                |             | R       | C | SWS2B |
| Field  | FZ9       | 10736408                       | 14826       | 560               | 1488        | 1037           | 1901           | 44          | 74.62      | 25.38 | 100  | 33.29  | 15.65  | 50.08  | 0.97 |
|        | FZ10      | 9975040                        | 23638       | 1390              | 1876        | 1894           | 2376           | 0           | 75.77      | 24.23 | 100  | 30.52  | 26.33  | 43.15  | 0.00 |
|        | FZ11      | 6864530                        | 15196       | 798               | 654         | 458            | 1170           | 14          | 83.04      | 16.96 | 100  | 28.49  | 22.01  | 48.90  | 0.60 |
|        | LLIT      | 22709442                       | 136187      | 18897             | 26723       | 3429           | 22709          | 3014        | 79.07      | 20.93 | 100  | 47.86  | 5.38   | 41.44  | 5.32 |
|        | Mean      | 12571355                       | 4706.50     | 5411.25           | 7685.25     | 1704.50        | 8751.67        | 768.00      | 78.13      | 21.87 | 100  | 35.04  | 17.34  | 45.89  | 1.72 |
|        | Se        | 3481577.01                     | 29879.20    | 4498.64           | 6351.03     | 646.09         | 5229.22        | 748.72      | 1.89       | 1.89   | -    | 4.39   | 4.55   | 2.12   | 1.22 |
| Aquarium| LY        | 21584148                       | 273003      | 40177             | 82429       | 18303          | 35983          | 52          | 60.61      | 39.39 | 100  | 60.27  | 11.87  | 27.83  | 0.04 |
|        | SQ        | 21581834                       | 272743      | 34687             | 79679       | 28871          | 34329          | 212         | 60.47      | 39.53 | 100  | 55.69  | 6.19   | 37.97  | 0.15 |
|        | PE        | 24905208                       | 305271      | 40809             | 81017       | 20063          | 26989          | 124         | 64.30      | 35.70 | 100  | 63.20  | 14.43  | 22.28  | 0.10 |
|        | NE        | 20168834                       | 387049      | 66207             | 168363      | 22443          | 79481          | 1170        | 53.34      | 46.66 | 100  | 62.03  | 16.11  | 21.44  | 0.43 |
|        | Mean      | 22060006                       | 309516.50   | 45470.00          | 102872      | 22420          | 44195.50       | 389.50      | 59.68      | 40.32 | 100  | 60.30  | 17.34  | 45.89  | 0.18 |
|        | S.e.      | 1005269.25                     | 26948.78    | 7047.68           | 21837.55    | 2311.58        | 11923.10       | 262.22      | 2.29       | 2.29   | -    | 1.65   | 2.17   | 3.81   | 0.09 |
Table S3. Predicted *R. aculeatus* visual pigment peak spectral sensitivities (λ_max) compared to reference visual pigments (*O. latipes* RH1; *Oreochromis niloticus* SWS2B, RH2B, RH2Aalpha, LWS), *R. aculeatus* λ_max determined via MSP, and tuning sites and effects considered for predictions. ¹(Matsumoto et al., 2006), ²(Spady et al., 2006), ³(Dungan et al., 2016), ⁴(Fasick and Robinson, 1998), ⁵(Yokoyama et al., 2007), ⁶(Yokoyama and Tada, 2003), ⁷(Chinen et al., 2005), ⁸(Yokoyama and Jia, 2020), ⁹(Cheney et al., 2013)

|                  | RH1  | SWS2B | RH2C-1 | RH2C-2 | RH2A | LWS  |
|------------------|------|-------|--------|--------|------|------|
| Similarity to reference amino acid sequence (%) | 94.1 | 86.1  | 85.5   | 85.5   | 91.5 | 89.9 |
| Total variable amino acid | 21   | 49    | 51     | 51     | 30   | 36   |
| Variable amino acid in transmembrane regions | 15   | 32    | 30     | 29     | 18   | 19   |
| Variable amino acids at known tuning sites | 1    | 5     | 12     | 11     | 8    | 0    |
| Reference pigment peak absorbance (nm λ_max) | 502¹ | 425²  | 472²   | 528²   | 560² |      |
| Known tuning sites and applied tuning effects (nm) | S299 A (-2)¹,²,⁴, | F46V (+8)³, | A109G (-2)³, | G164A (+1)⁵, | W265T (-29)³, | M88C (+3)¹, | I112V (+1)³, | T266V (-2)³, | M88C (+3)¹, | I112V (+1)³, | C88A (-3)¹, | I112V (+1)³, |      |
| Candidate tuning sites – no effects documented | S166 A | C163F | C168F | S168A | C98A | V185C | C98A | V185C | A151T |      |
| Predicted peak absorbance (nm λ_max) | 500   | 403   | 474    | 476    | 526  | 560  |
| MSP peak absorbance (nm λ_max) | 498⁶  | 413³  | 480⁶   | 528⁷   |      |      |

*RH1, SWS2B, RH2C-1, RH2C-2, RH2A, and LWS are visual pigments.*
Table S4. Summary of stimuli presented to each fish and in which order, whether the fish was trained to receive a food reward from the Distractor (11 cpd) or the test gratings (0.5-6 cpd), the total number of trials conducted by each fish for each colour (total number of trials = 2438) and calculated discrimination thresholds at 62% correct choice. NA indicates not tested due to time taken to complete treatment 1.

| Fish ID | Size (SL, cm) | Treatment 1 | S +ve Threshold (cpd) | Treatment 2 | S +ve Threshold (cpd) |
|---------|---------------|-------------|-----------------------|-------------|-----------------------|
| Billy   | 16            | Green-yellow (179) | Control (11 cpd) | 2.19 | NA | NA | NA |
| Bitey   | 10            | Pink-purple (120) | Grating | 2.45 | Green-yellow (167) | Grating | 2.17 |
| Diego   | 16            | Achromatic (203) | Grating | 4.89 | Pink-purple (75) | Grating | 2.73 |
| Ernie   | 17            | Achromatic (205) | Control (11 cpd) | 5.30 | NA | NA | NA |
| Gilbert | 16.5          | Green-yellow (184) | Grating | 2.71 | Achromatic (235) | Grating | 5.03 |
| Lyra    | 10            | Pink-purple (120) | Grating | 2.95 | Green-yellow (167) | Grating | 3.04 |
| Mike    | 15            | Green-yellow (188) | Grating | 2.58 | Pink-purple (167) | Grating | 3.27 |
| Sophie  | 15            | Pink-purple (193) | Control (11 cpd) | 5.44 | Achromatic (235) | Control (11 cpd) | 5.44 |
Further references

Cheney, K. L., Newport, C., McClure, E. C. and Marshall, N. J. (2013). Colour vision and response bias in a coral reef fish. *J Exp Biol* 216, 2967-2973.

Chinen, A., Matsumoto, Y. and Kawamura, S. (2005). Reconstitution of ancestral green visual pigments of zebrafish and molecular mechanism of their spectral differentiation. *Mol Biol Evol* 22, 1001-10.

Dungan, S. Z., Kosyakov, A. and Chang, B. S. W. (2016). Spectral tuning of killer whale (orcinus orca) rhodopsin: Evidence for positive selection and functional adaptation in a cetacean visual pigment. *Mol Biol Evol* 33, 323-336.

Fasick, J. I. and Robinson, P. R. (1998). Mechanism of spectral tuning in the dolphin visual pigments. *Biochemistry* 37, 433-438.

Matsumoto, Y., Fukamachi, S., Mitani, H. and Kawamura, S. (2006). Functional characterization of visual opsin repertoire in medaka (oryzias latipes). *Gene* 371, 268-278.

Spady, T. C., Parry, J. W., Robinson, P. R., Hunt, D. M., Bowmaker, J. K. and Carleton, K. L. (2006). Evolution of the cichlid visual palette through ontogenetic subfunctionalization of the opsin gene arrays. *Mol Biol Evol* 23, 1538-47.

Yokoyama, S. and Jia, H. (2020). Origin and adaptation of green-sensitive (rh2) pigments in vertebrates. *FEBS Open Bio* 10, 873-882.

Yokoyama, S. and Tada, T. (2003). The spectral tuning in the short wavelength-sensitive type 2 pigments. *Gene* 306.

Yokoyama, S., Takenaka, N. and Blow, N. (2007). A novel spectral tuning in the short wavelength-sensitive (sws1 and sws2) pigments of bluefin killfish (lucania goodei). *Gene* 396, 196-202.