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Stochastic spineless expression creates the retinal mosaic for colour vision

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Abstract

Drosophila colour vision is achieved by R7 and R8 photoreceptor cells present in every ommatidium. The fly retina contains two types of ommatidia, called 'pale' and 'yellow', defined by different rhodopsin pairs expressed in R7 and R8 cells. Similar to the human cone photoreceptors, these ommatidial subtypes are distributed stochastically in the retina. The choice between pale versus yellow ommatidia is made in R7 cells, which then impose their fate onto R8. Here we report that the *Drosophila* dioxin receptor Spineless is both necessary and sufficient for the formation of the ommatidial mosaic. A short burst of spineless expression at mid-pupation in a large subset of R7 cells precedes rhodopsin expression. In *spineless* mutants, all R7 and most R8 cells adopt the pale fate, whereas overexpression of *spineless* is sufficient to induce the yellow R7 fate. Therefore, this study suggests that the entire retinal mosaic required for colour vision is defined by the stochastic expression of a single transcription factor, Spineless.

The ability to discriminate between colours has evolved independently in vertebrates and invertebrates^{1,2}. However, despite the obvious differences in eye development and design, both flies and humans have developed retinal mosaics where classes of photoreceptor cells (PRs) with different spectral sensitivity are randomly distributed^{3,4}.

The compound eye of *Drosophila* consists of ~800 optical units (ommatidia), each containing eight PRs in addition to accessory cells⁵. In each ommatidium, the six 'outer PRs' (R1–R6) function like the vertebrate rod cells, as they are required for motion detection in dim light^{6,7}. These cells express the broad-spectrum rhodopsin, Rh1 (ref. 8). The 'inner PRs' (R7 and R8) may be viewed as the equivalent of the colour-sensitive vertebrate cone cells, which express a range of different rhodopsin molecules^{9–13}.

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Ommatidial subset specification in *Drosophila*

The general rule of sensory receptor exclusion also applies to *Drosophila* ommatidia, where only one rhodopsin gene is expressed by a given PR¹⁴. The expression of inner PR rhodopsins can be used to distinguish three ommatidial subtypes^{15,16} (Supplementary Fig. 1a, b). Two of the subtypes are distributed randomly throughout the retina: ~30% of ommatidia express ultraviolet-sensitive *Rh3* in R7 cells and blue-sensitive *Rh5* in R8 cells, and therefore are specialized in the detection of short wavelengths ('pale' ommatidia, p; Fig. 1a, blue). The remaining ~70% express another ultraviolet-sensitive opsin (*Rh4*) in R7 and green-sensitive *Rh6* in R8, making them more responsive to longer wavelengths ('yellow' ommatidia, y; Fig. 1a, yellow). The coupled expression of *Rh3/Rh5* or *Rh4/Rh6* within the same ommatidium results from communication between R7 and R8 (Supplementary Fig. 1b, c). In the dorsal rim area (DRA) (Fig. 1a, pink), a third type of ommatidia exists¹⁷ in which both R7 and R8 express ultraviolet-sensitive *Rh3* (refs 18, 19). These ommatidia are used to detect the e-vector of polarized sunlight for orientation^{20,21}. Spatially localized polarized light detectors and stochastically distributed colour-sensitive ommatidia therefore reflect two fundamentally different specification strategies that shape the retinal mosaic of *Drosophila*²².

The current model for specifying colour-sensitive ommatidia combines stochastic and instructive steps¹¹. First, a subset of R7 (pale R7, pR7) stochastically chooses *Rh3* expression over the 'R7 default', *Rh4*. Second, these cells then impose the p fate (*Rh5*) onto R8 (pale R8, pR8) of the same ommatidium (Supplementary Fig. 1c).

In this study, we report the identification of *spineless* (*ss*) as a key regulatory gene for establishing the retinal mosaic. *ss* encodes the *Drosophila* homologue of the human arylhydrocarbon ('dioxin') receptor, a member of the bHLH-PAS (basic helix-loop-helix-Period-Arnt-Single-minded) family of transcription factors^{23,24}. At mid-pupation, *ss* is stochastically expressed in a majority of R7 that seem to correspond to the y subtype. *ss* is both necessary and sufficient to specify the yellow R7 (yR7) fate and subsequently the entire y ommatidia; pR7 cells are thus specified by default, and stochastic expression of *ss* represents the key regulatory event defining the retinal mosaic required for fly colour vision.

***spineless* is necessary for yellow ommatidia specification**

We recently identified *homothorax* (*hth*) as the key regulatory gene necessary and sufficient for the specification of DRA ommatidia¹⁹. *ss* and *hth* cause similar homeotic phenotypes: that is, complete (*hth*) or partial (*ss*, 'aristapedia') transformation of antennae into legs^{25,26}. Therefore, we tested for a potential role of *ss* in ommatidial subtype specification by generating whole-mutant eyes, as well as mitotic clones, lacking *ss* function using the null allele *ss*^{D115.7} and the *ey*-FLP/FRT technique^{27,28}. Owing to *ey*-FLP expression in the antennal imaginal disc, *ss* mutant flies showed a strong *aristapedia* phenotype (Supplementary Fig. 2a), but lacked any obvious morphological eye phenotype. However, expression of rhodopsin genes was severely affected. In wild-type eyes, Rh3 is found in ~30% of R7 cells, as well as in both R7 and R8 of DRA ommatidia (Fig. 1c, arrow), whereas the remaining ~70% of R7 contain Rh4 (Fig. 1c). In *ss* mutant eyes, Rh4 was completely absent, whereas Rh3 was expanded into all R7 cells (Fig. 1d). The total number of ommatidia was not reduced, indicating that R7 cells were mis-specified into pR7, rather than yR7 being specifically eliminated. *ss* mutant mitotic clones were morphologically wild type (Supplementary Fig. 2b); however, Rh3 was always present in mutant R7 cells (marked by the absence of -galactosidase (-gal) expression), whereas Rh4 was always lost (Fig. 1e).

To test whether the R7 *ss* phenotype was cell autonomous, we generated individual mutant R7 cells using the MARCM technique^{29,30}. All mutant R7 cells (marked by the presence of green fluorescent protein (GFP) expression) contained Rh3 and never Rh4, demonstrating that *ss* is required cell autonomously in R7 to induce Rh4 expression (Fig. 1f). DRA ommatidia were correctly specified in *ss* mutant eyes, as Rh3 was expressed normally in both DRA R7 and R8 cells (Fig. 1d, arrow). Therefore, *ss* is necessary for the establishment of the yR7 subtype without affecting PR fate specification (Fig. 1b and Supplementary Fig. 2c).

The ommatidial subtypes are first specified in R7, which then instruct R8 (ref. 16). Therefore, *ss* mutant eyes should exhibit a rhodopsin phenotype in R8. In wild types, ~30% of R8 cells contain Rh5, and the remaining ~70% contain Rh6 (Fig. 2c)^{11,12}. In *ss* mutant eyes, the large majority (up to 95%) of R8 contained Rh5 (Fig. 2a, d), with some R8 still containing Rh6. However, most of these remaining yR8 cells were located in the dorsal third of the eye (Fig. 2a). In this part of the retina, instruction of pale R8 (pR8) by pR7 is less efficient, resulting in ommatidia with odd-coupled (Rh3/Rh6) rhodopsin expression (E.O.M. & C.D., manuscript in preparation). In *ss* mutants, the frequency of such ommatidia was significantly increased in the dorsal region (Fig. 2b).

To test whether the R8 opsin phenotype of *ss* mutants resulted from the inability of some mutant R7 cells to properly instruct R8, rather than from *ss* being directly required in R8, we generated *sevenless*; *spineless* (*sev*; *ss*) double-mutant eyes. These eyes, which lacked R7 cells, always exhibited the *sev* single-mutant phenotype (Fig. 2e), with virtually all R8 cells containing Rh6 (Fig. 2f). This indicates that *ss* is required in R7 for the formation of the yR7 subtype, and consequently for the formation of yR8, without being directly required in R8 PRs.

***spineless* induces the yellow ommatidial subtype**

We tested whether *ss* was also sufficient to induce the y ommatidial subtype (Fig. 3a). Overexpression of *ss* in all developing PRs using a strong *LGMR* (long *glass* multiple reporter)-Gal4 driver³¹ and *UAS-Ss* (*LGMR>ss* flies) resulted in a rough eye phenotype, as well as a dramatic rhodopsin phenotype: *Rh4* was activated in all PRs throughout the eye (R1–R6 as well as R7 and R8), as revealed by ectopic expression of an *Rh4*-GFP reporter in many PRs per ommatidium (Fig. 3c) compared with wild type (Fig. 3b). To avoid the strong phenotype in the eye, we misexpressed *ss* using the weaker, variegated GMR driver, *sGMR* (short GMR)-Gal4³¹ (*sGMR>ss* flies). This led to strong ectopic induction of Rh4 in many PRs without severely affecting retinal morphology (Fig. 3d). This ectopic induction of Rh4 was also observed in *sev* mutants (Fig. 3f), and was thus independent of R7. Rh3 was still detected in some R7 in *sGMR>ss* flies, presumably due to the lack of variegated Gal4 expression in these cells (Fig. 3g, arrows), whereas Rh4 was expanded to some outer PRs. However, co-localization of Rh3 and Rh4 was never observed, confirming that gain of Rh4 in R7 cells always leads to the exclusion of Rh3. In contrast, gain of Rh4 in outer PRs did not lead to the exclusion of Rh1, as frequent coexpression of Rh1 and Rh4 was observed (Fig. 3i, arrows).

Using an *Rh4-lacZ* reporter construct in *LGMR>ss* flies, we found that -gal-positive PR axons projected to both lamina and medulla, confirming the expansion of *Rh4* into outer PRs (Supplementary Fig. 3a). However, *Rh4*-expressing outer PRs were not transformed into genuine R7 cells, as they maintained their lamina projections (Supplementary Fig. 3a). Notably, DRA inner PRs were the only cells not expressing *Rh4* (Supplementary Fig. 3a), suggesting that the DRA fate^{19,32}, specified by the gene *hth*, antagonizes *ss* function. Expression of *Rh3* and *Rh5* was completely lost (including in the DRA, where no rhodopsin

was detected), while *Rh6* expression was found in most R8 cells (Supplementary Fig. 3b). This resulted in R8 coexpressing *Rh4* and *Rh6*, demonstrating that the ‘one sensory receptor per cell’ rule can be broken in *Drosophila* PRs, as has been shown in other insects^{14,33}. Therefore, ectopic induction of the yR7 fate by *ss* specifically excludes the formation of pR7 cells. As a consequence, R8 cells expressing *Rh5* are not induced¹⁶, with most R8 expressing *Rh6*. *Rh6* was never found in outer PRs, supporting the hypothesis that *ss* is required only in R7 for the choice between *Rh3* and *Rh4*, and not directly in R8 for the *Rh6* choice (Supplementary Fig. 3b). In *LGMR>ss* flies, the specification of outer versus inner PRs (markers *spalt* and *seven up*; Supplementary Fig. 3c) or of R7 versus R8 (*prospero* and *senseless*; Supplementary Fig. 3d) was normal. Thus, *ss* acts by segregating ommatidial subtypes downstream of early PR specification events.

***spineless* can re-specify cell fate in R7 photoreceptor cells**

Colour PR cell fate determination seems to be a late event in PR differentiation. To test whether *ss* can transform the R7 fate at late stages of development, we used the *PanR7-Gal4* driver (which is also expressed in DRA R8 cells). Late mis-expression of *ss* induced the y fate (Rh4) in all R7 cells, whereas Rh3 was absent (Fig. 4a, left panel). Opsin expression in the DRA was also altered, with Homothorax-positive cells (both R7 and R8) now expressing Rh4 (Supplementary Fig. 4). Hence, it is possible to reprogramme the R7 fate at later stages of differentiation, as *PanR7-Gal4* becomes activated at the time of rhodopsin expression. Surprisingly, expression of R8 rhodopsins outside the DRA was not affected, as the distribution of Rh5 and Rh6 resembled the wild type (Fig. 4a, right panel). As a result, many ommatidia manifested the very unusual coupling of Rh4 in R7 and Rh5 in R8 (Fig. 4b). Therefore, although *ss* is able to reprogramme all R7 late in development, R8 cannot revert their fate once they have been instructed to become pR8, and they maintain Rh5. We have recently identified two antagonistic genes expressed in either of the two R8 subtypes, which act together as a molecular consolidation system responsible for this inertia of R8 (ref. 34). To confirm that late expression of *ss* exclusively in R7 was sufficient to transform R7, *ss* was mis-expressed in *ss^{D115.7}* mutants using *PanR7-Gal4*. This was sufficient to induce Rh4 and to repress Rh3 (Fig. 4c, left panel). R8 were again not reprogrammed and exhibited the *ss* mutant phenotype, with many R8 cells expressing Rh5 (Fig. 4c, right panel).

Transient expression of *spineless* precedes R7 specification

All of the results presented above strongly indicated that *ss* must be expressed in the y subtype of R7 at some point during pupal development. As several attempts to generate an anti-Spineless antibody had failed, we used *in situ* hybridization to detect *ss* messenger RNA in the retina at mid-pupation (Fig. 5a, b). At ~50% pupation, *ss* mRNA was detected in four neuronal cells per ommatidial cluster, one PR and three bristle cells (Fig. 5a, circles and asterisks). The PR was also labelled by anti-Prospero, confirming its identity as R7 (Fig. 5b). Although the expression levels of *ss* in bristle cells seemed uniformly high, levels of *ss* expression varied considerably among R7 cells, ranging from very faint to very strong in 60–80% of R7.

We also identified a 1.6 kilobase ‘eye enhancer’ fragment (*ss_{eye}*) within the *ss* promoter that drives PR-specific expression. After crossing *ss_{eye}-Gal4* to *UAS-gal::NLS* (nuclear localization sequence) reporters, PR-specific *ss* expression was first detected at mid-pupation—that is, approximately one day before rhodopsins are expressed, and before any visible molecular or morphological distinction between ommatidial subtypes (Fig. 5c). A single PR per ommatidium, which was identified as R7 through co-staining with Prospero, expressed *ss* (Supplementary Fig. 5a). Thus, the *ss_{eye}* enhancer recapitulates endogenous *ss* expression in PRs. *ss* expression was detected in 60–80% of R7, correlating well with the

distribution of Rh4 in adult retina. Like Rh4-expressing ommatidia, *UAS*-*Gal4*-positive ommatidia were more abundant in the dorsal half of the eye, and no *UAS*-*Gal4* expression was detected in the DRA (marked by *Homothorax*), where Rh4 is also never expressed (Supplementary Fig. 5b). *ss_{eye}*-*Gal4* expression was detectable for only ~2 h at midpupation. Although it was not possible to directly co-stain for *ss* and Rh4 (which starts to be expressed one day later during pupation), it seems that at mid-pupation a short pulse of *ss* is deployed in a large subset of R7, which will become yR7.

***spineless* levels are crucial for specifying yellow ommatidia**

We tested whether a short pulse of ectopic *ss* expression was able to modify the entire retinal mosaic, using a *heat shock*-*Gal4* driver (*hs*-*Gal4*) to temporally control *ss* expression (*hs*>*ss* flies). A 30-min heat shock at ~50% pupation indeed resulted in an increase of Rh4 expression with a concomitant reduction of Rh3 in adults. The phenotype varied extensively, from only R7 cells expressing Rh4 (~25% of the flies analysed had Rh4 in most R7) (Fig. 6a), to almost every PR expressing Rh4 (Fig. 6b). In contrast, a 30-min pulse of *ss* in one-day-old adult flies had no effect (data not shown). Heat shocks during larval or early pupal stages were lethal. Thus, PRs are extremely sensitive to a short pulse of *ss* during mid-pupation, at the time when endogenous *ss* is normally expressed.

To further study the mechanism of the stochastic choice between p and y ommatidia, we analysed the retinal mosaic in different mutant backgrounds. Flies heterozygous for *ss^{D115.7}* had fewer Rh4-expressing R7 cells (control: $66.1 \pm 3\%$; *ss* heterozygous: $53.8 \pm 3.5\%$; $P < 0.001$; values are mean \pm s.d.). As the *ss^{D115.7}* allele only affects the *ss* coding sequence, heterozygous flies have two functional promoters, only one of which produces a functional protein, suggesting that the non-productive promoter might sequester limiting factor(s) that regulate(s) the expression levels of *ss*. If this hypothesis is correct, addition of extra copies of the *ss* promoter should have a similar effect. Indeed, the addition of two functional copies of the *ss* eye enhancer (*ss_{eye}*-*Gal4*) in an otherwise wild-type background also caused a significant reduction of the yR7 subtype ($50.2 \pm 2.4\%$, $P < 0.001$). Therefore, the level of Spineless expression is important for the induction of the yR7 fate, which is less efficient in cells where the amount of Spineless is reduced.

Conclusions

Retinal patterning in *Drosophila* reveals an original mechanism for how PR mosaics can be generated: stochastic expression of a single transcription factor (Spineless) acts as a binary switch that transforms the seemingly homogeneous compound eye into a mosaic, distinguishing p and y subtypes. However, subtype specification and rhodopsin expression can be separated, as *ss* expression in yR7 has ceased well before the time of rhodopsin expression (Fig. 6c). Additional factors are therefore required downstream of *ss* to ensure expression of adult p- and y-specific markers such as rhodopsins and additional screening pigments⁴. We propose a revised two-step model for the stochastic specification of p and y ommatidia (Fig. 6d). First, R7 are stochastically divided into two subtypes by the induction of *ss* in yR7. *ss*-positive R7 express *Rh4*, whereas the remaining R7 choose the pR7 fate and express *Rh3* by default (Fig. 6d, left). Second, only those R7 cells that did not express *ss* (pR7) retain the ability to induce the pR8 fate (*Rh5*), whereas yR8 express *Rh6* by default (Fig. 6d, right). The ‘default states’ of R7 (*Rh3*) and R8 (*Rh6*) therefore belong to opposite subtypes. Expression of R8 rhodopsin genes is maintained by a bistable regulatory loop containing the genes *warts* and *meltec²*⁴. Notably, the localized specification of polarization-sensitive DRA ommatidia by *hth* antagonizes the stochastic choice executed by *ss*, placing these two genes into a new regulatory relationship during retinal patterning. Therefore, the role of the transcription factor Spineless is to generate the retinal mosaic

required for fly colour vision by distinguishing yR7 from pR7 cell fates, and preventing R7 from instructing the underlying R8 cells.

Mosaic expression of sensory receptors has been described in detail for the olfactory system of both vertebrates³⁵ and insects³⁶, and random PR mosaics have been described for humans³ and amphibians³⁷, as well as insects^{4,15,38–40}. Two transcription factors have been shown to regulate the specification of blue versus red/green cone cell fates in mammals. Upon mutation of either—the human nuclear receptor NR2E3 (also known as PNR) or the rodent thyroid hormone β 2 receptor—the number of blue cones is dramatically increased at the expense of green cones^{41,42}, leading to ‘enhanced S-cone syndrome’. It should be noted that this retinal phenotype bears important similarity to the altered ommatidial mosaic in *Drosophila ss* mutants, where long wavelength-sensitive y ommatidia are lost at the expense of the short wavelength-sensitive p type.

The stochastic cell fate choice occurs at the level of the *ss* promoter: the very short pulse of *ss* expression at mid-pupation is not only controlled temporally, but its levels are also critical, and only ~70% of R7 receive enough Spineless to commit to the yR7 fate. Elucidating the mechanism that controls *ss* expression will shed some light into the fascinating process of stochastic gene expression, and the identification of its downstream targets will provide insights into consolidation and maintenance of cell fates.

METHODS

Drosophila strains and crosses, constructs, generation of transgenic flies by germ line transformation, antibody staining on mid-pupal and adult whole-mounted or cryo-sectioned retinas, *in situ* hybridization on pupal retinas, MARCM, cornea neutralization, and adult eye plastic sections were all performed by standard methods, and are described in detail in Supplementary Information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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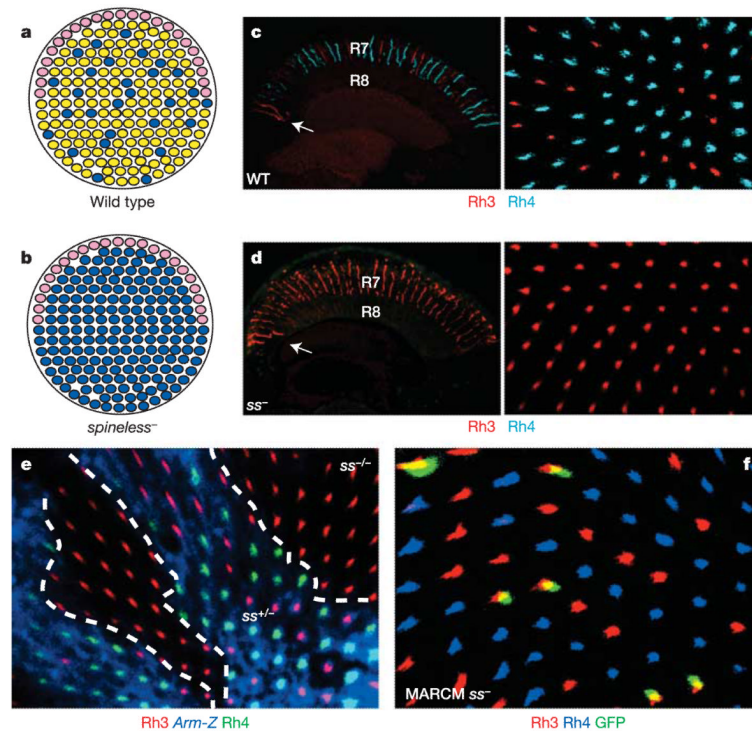


Figure 1. The yR7 subtype is lost in *spineless* mutants

a, Three subtypes can be identified on the basis of molecular markers: ‘pale’ (blue), ‘yellow’ (yellow) and DRA (pink) ommatidia together form the wild-type retinal mosaic (schematic representation; dorsal is to the top). **b**, Schematic representation of the *ss* phenotype in R7 cells. **c**, Transverse section through a wild-type (WT) adult eye (left panel; dorsal is to the left). The arrow denotes the DRA. Ratio of R7 opsins in a wild-type whole-mount adult retina (right panel; dorsal is to the top) stained for Rh3 (red) and Rh4 (cyan). **d**, Transverse section through a *ss^{D115.7}* whole-mutant adult eye (left panel). Rh3 (red) is expanded and Rh4 (cyan) is completely lost. Opsin expression in a mutant whole-mount adult retina is also shown (right panel). **e**, Whole-mount retina with mitotic clones lacking Spineless (marked by the absence of expression of the *armadillo-lacZ* (*Arm-Z*) construct, blue). Rh4 (green) is always absent from mutant clones. Rh3 expression is shown in red. **f**, Whole-mount retina with MARCM clones lacking Spineless (marked by the presence of GFP, green). Every GFP-positive cell expresses Rh3 (red), whereas Rh4 (blue) is always absent from mutant cells.

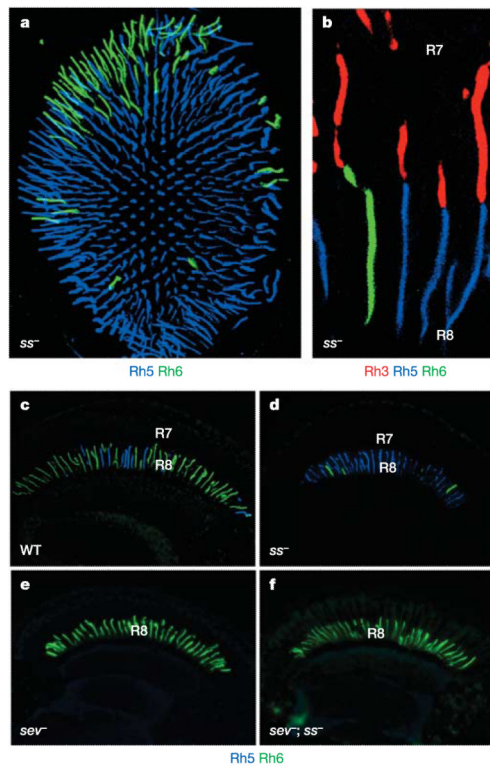


Figure 2. R8 phenotype of *spineless* mutant eyes

a, Whole-mount retina from a *ss* mutant fly. The pR8 subtype (Rh5, blue) is expanded to almost all R8 cells (Rh6, green). **b**, Unusual mis-coupling of Rh3 (red) in R7 and Rh6 (green) in R8 of the same ommatidium is frequently observed in *ss* mutant retinas. Rh5 expression is shown in blue. **c–f**, Transverse sections stained for Rh5 (blue) and Rh6 (green): an adult wild-type eye (**c**), a *ss* mutant eye (**d**), a *sev* mutant eye (**e**), and a double-mutant (*sev*; *ss*) eye (**f**), which manifests the same R8 phenotype as *sev* mutants (expansion of yR8 cells and loss of pR8 cells).

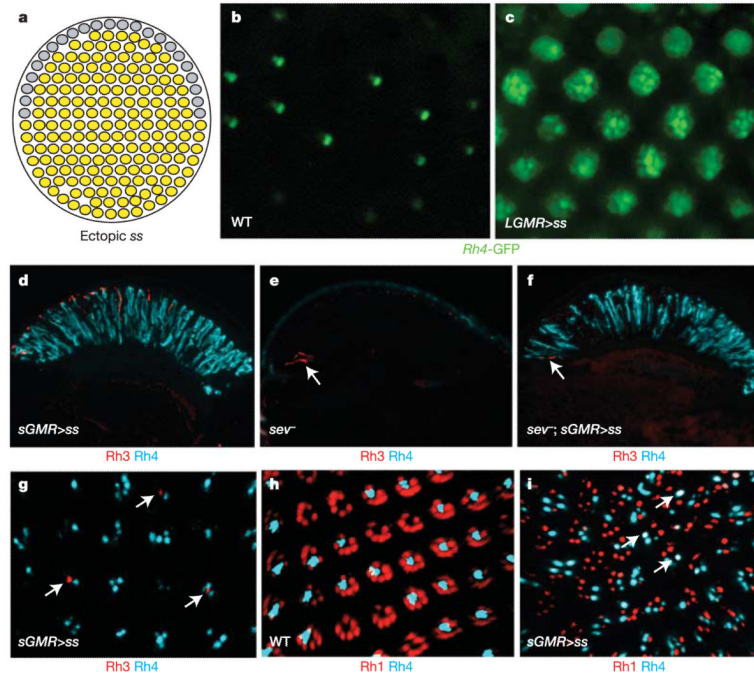


Figure 3. *spineless* is sufficient to induce the yR7 fate

a, Summary of the *ss* gain-of-function phenotype. All R7 cells adopt the yR7 fate (yellow); the fate of DRA ommatidia is unclear (grey). **b**, Water immersion microscopy on living wild-type flies expressing the yR7-specific reporter *Rh4-GFP*. Expression is restricted to one inner PR in a large subset of ommatidia. **c**, *Rh4*-driven GFP expression is dramatically expanded in *LGMR>ss* flies, as visualized by water immersion. **d**, In *sGMR>ss* flies, Rh4 (cyan) is expanded through the whole *ss*-overexpressing retina (compare with Fig. 1c). **e**, Rh4 (cyan) is completely lost in *sev* mutants, although Rh3 (red) is present in DRA R8 cells (arrow). **f**, Overexpression of *ss* (*sGMR>ss*) leads to ectopic Rh4 (cyan) in *sev* mutants. Rh3 (red) is restricted to DRA R8 cells (arrow). **g**, In whole-mount retinas from *sGMR>ss* flies, variegated expression of *ss* leads to the expansion of Rh4 (cyan) into a variable number of PRs per ommatidium; coexpression with Rh3 (red) is never observed (arrows). **h**, In wild-type eyes, the outer-PR opsin Rh1 (red) and Rh4 (cyan) never co-localize in the same PR cell. **i**, Ectopic expression of *ss* in *sGMR>ss* flies leads to massive expansion of Rh4 (cyan), with some co-localization (arrows) with the outer-PR opsin Rh1 (red).

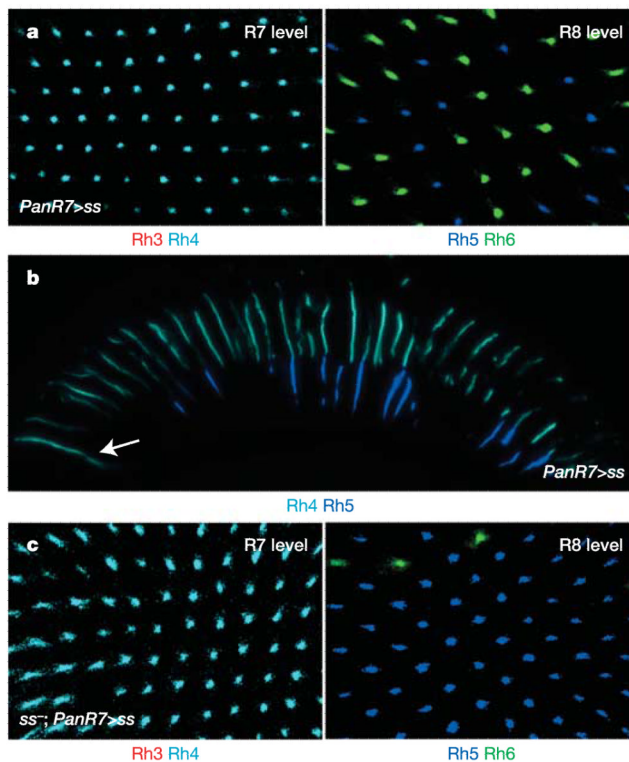


Figure 4. Control of PR cell fates by *spineless*

a, In whole-mount retinas from *PanR7>ss* flies, all R7 cells express Rh4 (cyan), whereas Rh3 (red) is absent (left panel). Late expression of *ss* in R7 has no effect on the ratio between Rh5 (blue) and Rh6 (green) expression (right panel). **b**, Section of a *PanR7>ss* eye. As the fate of R8 is not affected by late *ss* expression in R7 cells, there is a great number of ‘odd-coupled’ Rh4 (cyan)/Rh5 (blue) ommatidia. The arrow indicates the DRA. **c**, In whole-mount retinas from *PanR7>ss* flies on a *ss* mutant background, Rh3 (red) is not expressed, while every R7 cell expresses Rh4 (cyan) (left panel). Late overexpression of *ss* in all R7 cells on a *ss* mutant background does not lead to a reprogramming of R8 cells as most R8 cells express Rh5 (blue) and few dorsal R8 express Rh6 (green) (right panel).

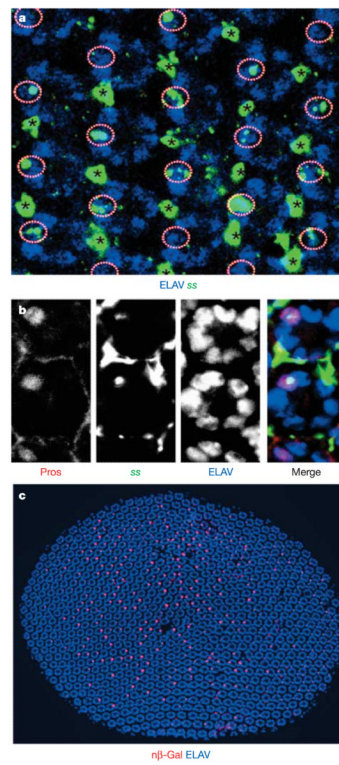


Figure 5. Mosaic expression of *spineless* in the developing retina

a, *In situ* hybridization of a whole-mount pupal retina with an antisense *ss* probe (green) and an ELAV antibody (blue). *ss* expression can be observed in all bristle cells (asterisks), as well as in one PR (circles) per ommatidium in only 60–80% of all ommatidia. Note that *ss* levels vary from cell to cell. **b**, The PR cell positive for *ss* (green) is identified as an R7 cell by co-staining with the R7-specific marker Prospero (Pros, red). Neurons are marked with ELAV (blue). **c**, Nuclear β -gal (n -Gal, red) driven under the control of *ss_{eye}*-Gal4 reveals mosaic expression of *ss* in one cell per cluster. Neurons are marked with ELAV (blue). Dorsal is to the left in all panels.

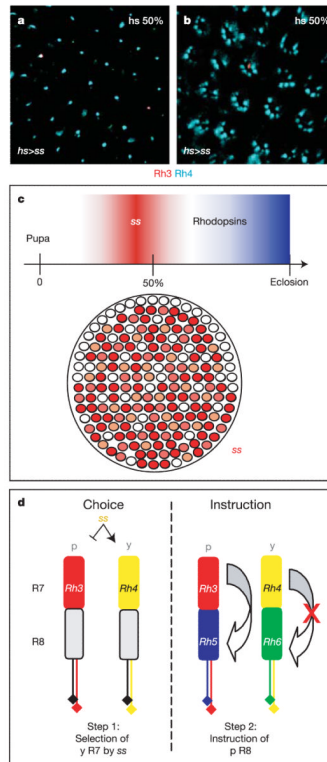


Figure 6. Stochasticity of *ss* expression and a revised model for retinal patterning in *Drosophila*
a, b, The effect of a 30-min pulse of *ss* expression at ~50% pupation leads to an almost 100% transformation of R7 to the y fate (**a**), or almost every PR (**b**). **c**, Top: transient expression of *ss_{eye}-Gal4* (red) during pupation before the onset of opsin expression (blue). Bottom: variable expression of *ss* (different tones of red) in R7 cells. **d**, Left: the *ss* data suggest that ~70% of the R7 cells get promoted into the yR7 fate (*Rh4*, yellow) by expressing *ss*. The pR7 subtype (*Rh3*, red) therefore represents the R7 'default state'. Right: in *ss*-positive yR7 cells, the ability to communicate with the underlying R8 is abolished, resulting in y ommatidia as the R8 default state is expression of *Rh6* (green). Only pR7 retain the competence to instruct the pR8 fate (*Rh5*, blue).