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Behavioural changes in slime moulds over time

**Rolland Angèle^{1†}, Pasquier Emilie^{1†}, Malvezin Paul^{1†}, Cassandra Craig^{1†},
Dumas Mathilde[†], Dussutour A[†]**

[†]Research Centre on Animal Cognition (CRCA), Centre for Integrative Biology (CBI), Toulouse University, CNRS, UPS, Toulouse 31062, France.

¹Contributed equally to the work

Keywords: Ageing, learning, decision making, *Physarum polycephalum*, slime moulds

Summary

Changes in behaviour over the lifetime of single cell organisms has primarily been investigated in response to environmental stressors. However, growing evidence suggests that unicellular organisms undergo behavioural changes throughout their lifetime independently of the external environment. Here we studied how behavioural performances across different tasks vary with age in the acellular slime mould *Physarum polycephalum*. We tested slime moulds aged from 1 week to 100 weeks. First, we showed that migration speed decreases with age in favourable and adverse environments. Second, we showed that decision making and learning abilities do not deteriorate with age. Third, we revealed that old slime moulds can recover their behavioural performances if they go throughout a dormant stage or if they fuse with a young congener. Lastly, we observed the response of slime mould facing a choice between cues released by clone mates of different age. We found that both old and young slime moulds are attracted toward cues left by young slime moulds. Although many studies have studied behaviour in unicellular organisms, few have taken the step of looking for changes in behaviour over the lifetime of individuals. This study extends our knowledge of the behavioural plasticity of single-celled organisms and establish slime moulds as a promising model to investigate the effect of aging on behaviour at the cellular level.

*Author for correspondence (audrey.dussutour@cnrs.fr).

[†]Present address: Research Centre on Animal Cognition, Centre for Integrative Biology, Toulouse University, CNRS, UPS, Toulouse 31062, France.

Introduction

One aim of this special issue of *Philosophical Transactions* is to understand collective behaviour over the lifetime of individuals. In unicellular organisms, changes in collective behaviour through time has been studied almost exclusively in response to environmental stressors. For instance, it has been shown that bacteria [1,2] and cellular slime moulds [3–5] transit to new collective states such as coordinated cellular migration or aggregation when nutrients become scarce [6]. On the contrary, how collective behaviour changes with correlates of age in unicellular organisms independently of the external environment remains an open question. The main reason for this might be that, single cell organisms were mistakenly believed to be short lived and immune to ageing under optimum growth conditions [7–11]. It has now been demonstrated that, some unicellular organisms such as bacteria *Escherichia coli* and *Caulobacter crescentus*, ciliates *Paramecium* and *Tetrahymena*, yeast *Saccharomyces cerevisiae*, cellular and acellular slime moulds *Dictyostelium discoideum* and *Physarum polycephalum* undergo intrinsic changes over time that affect cell behaviour and physiology [11–13]. In this paper, our aim is to understand how cell behaviour varies over the lifetime of individuals in the acellular slime mould *P. polycephalum*. In contrast to the cellular slime mould *D. discoideum* in which the vegetative state consists of solitary amoebas that divide about every 4 h when food is plentiful, in *P. polycephalum*, the vegetative state is a unique large cell containing a large collection of nuclei that divide about every 8 h and share a common cytoplasm. Acellular slime moulds are particularly attractive organisms to study collective behaviour changes over time in unicellular organisms for several reasons.

First of all, although they are not collective *per se*, acellular slime moulds embrace most of the key principles of collective behaviour (reviewed in [14]): synchronization and coordination [15–18], positive and negative feedbacks [19], variability [20–22], symmetry breaking [20,23], phase transition [24], redundancy [14], information transfer [25,26] capacity to make complex decisions [27–30]. In addition, due to its internal dynamic, *P. polycephalum* is often regarded as a collection of nonlinear oscillators which are interconnected, often coupled and partially dependent on each other (reviewed in [16]). Furthermore, similarly to more traditional model systems in collective behaviour such as ant colonies or fish schools, its behaviour has successfully been simulated using a multi-agent approach [31,32]. In these models, *P. polycephalum* behaviour relies on particle like agents that follow simple rules but are capable collectively to generate self-organized amoeboid movement and to construct optimized networks [31,32].

Second, despite being unicellular, acellular slime moulds can produce seemingly complex behaviours. Indeed, since the seminal contribution of Toshiyuki Nakagaki twenty years ago [33], *P. polycephalum* has become an essential model organism for studying problem-solving in non-neural systems [32–37]. Past experiments have shown that acellular slime moulds can find the shortest path in a maze [33,38], build optimized networks to connect several food sources [34], anticipate events

1 [39], learn to ignore irrelevant stimuli [40,41], encode memory in their environment [42] or in their
2 morphology [43], interact with their congeners [21,44], optimize nutrient intake [28,45], make optimal
3 decisions [29,46,47], etc. *P. polycephalum*'s behaviour relies on its self-organized internal architecture
4 which consists of a transport network of interconnected tubes [48]. The tubes contract and relax
5 periodically, causing the cytoplasm to flow back and forth, a phenomenon termed 'shuttle streaming'
6 [49]. *P. polycephalum* can migrate at a speed of up to few millimetres per hour through the interplay
7 of intracellular flow, adhesion and rhythmic contractions of the tubes [49–51]. The frequency and the
8 amplitude of the contractions depend on external stimuli and as a result, *P. polycephalum* is capable of
9 altering its shape and motion as a function of a variety of external stimuli such as chemicals [52–54],
10 light [55], temperature [56–58], humidity [44], gravity [59] or substrate distortion [46].

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Third, acellular slime moulds are easy to track through time and relatively long-lived. In unicellular organisms, lifetime can either be chronological if we consider the time a cell remains viable before dividing, or replicative if we consider the number of times a cell divide before it dies [9]. Tracking division is usually difficult as it requires to follow and distinguish individual cells. *P. polycephalum* offers the possibility to consider both chronological and replicative time simultaneously. During its development, the zygote undergoes synchronous mitotic divisions every 8 to 12 hours but without cytokinesis to form a single multinucleated cell, called a plasmodium [60,61]. Hence, the cell grows over time but there is no cell division. A plasmodium reared on solid medium by routine serial subculture under laboratory condition can be maintained from a few weeks to several months [62,63]. The life span of the plasmodium is in part genetically determined as different strains have different lifespans and subclones derived from a single plasmodium age in a coordinated manner, i.e death occurs at approximately the same time [62,64,65].

Fourth, *P. polycephalum* provides unique possibilities for experimental manipulations [66,67]. The plasmodium can extend up to hundreds of square centimetres. It can be severed into viable and structurally similar yet smaller plasmodia. Upon contact, genetically identical plasmodia can fuse with each other to form a single plasmodium. In response to environmental stressors, the plasmodium can enter a resting stage called sclerotium and remain dormant for years without deterioration. During the sclerotization process, the plasmodium lose 50% of their total protein content together with 40% of their DNA and 65% of their RNA [60]. After transfer to a humid and nutritive medium, a sclerotium can revert back to a mobile plasmodium within 24 h. Plasmodial cultures can be easily initiated from sclerotia after up to 3 years. Interestingly, numerous authors have reported that, a sclerotium issued from a seemingly ageing plasmodium may produce a vigorous plasmodium again, a form of "rejuvenescence". Following a period of growth this secondary plasmodium will undergo senescence again. This cycle of growth and senescence can be repeated several times and, thus, cultures can be maintained over many generations.

1 Lastly, *P. polycephalum* displays senescence, which is defined as a loss of fitness during ageing.
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3 A plasmodium can be maintained for months during which no change is apparent until a reproducible
4 decline in viability occurs [62]. Senescence in the plasmodial stage is characterized by morphological
5 change [62], reduction of growth rate [62,68], reduced cytoplasmic flow [64], loss of yellow pigment
6 [64], accumulation of polyploid nuclei [69], high level of mitochondrial DNA fragmentation [68],
7 decreased replication of mitochondrial DNA [63], increase in nuclear size and DNA content [69,70].
8 Senescence ends with the fragmentation of the plasmodium into several small spherical structures with
9 a concomitant lysis of the plasmodium [64]. Longevity in slime moulds is not affected by most
10 environmental factors with the exception of high temperature [71]. Senescence in slime moulds has
11 been mainly studied at the cellular level. The potential effects of ageing on the plasmodium behaviour
12 remained to be investigated.
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20 In this paper, we studied how slime mould behaviour changes over time. First, we explored the
21 effect of age on migration speed in nutritive and adverse environments. Second, we investigated if
22 decision making and learning performances changed with age. Third, we tested if older slime moulds
23 were able to recover after a dormant period or after fusing with younger clones. Lastly, we examined
24 if slime moulds were able to discriminate cues from congeners of different age.
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30 **Methods**

31 **(a) Species studied and rearing conditions**

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33 *Physarum polycephalum*, also called the acellular slime mould, belongs to the supergroup Amoebozoa
34 and the class Myxomycetes. In nature, slime moulds are found on organic substrates like tree bark or
35 forest soil where they feed on microorganisms such as bacteria or fungi. *P. polycephalum* vegetative
36 morph is a vast multinucleated cell named plasmodium. We used a strain of *P. polycephalum* : LU352
37 kindly provided by Professor Dr Wolfgang Marwan (Max Planck Institute for Dynamics of Complex
38 Technical Systems, Magdeburg, Germany). 80 large plasmodia were initiated from sclerotia at
39 different time to obtain slime moulds of different age. In this paper, by age of the slime mould, we
40 understand the time which elapses from the moment of the inoculation of a sclerotium onto a nutritive
41 medium. The oldest plasmodia were kept after this study and are still being cultivated in our laboratory.
42 They are currently more than 2 years old and none of them has died so far. In previous reports,
43 plasmodium lifespan has been shown to vary from one month to one year, depending on the strain [62-
44 64]. Hence, we considered as “old”, slime molds which were over one year old and “very old” slime
45 molds which were almost two years old.
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57 Plasmodia were reared on a 1% w/v (weight/volume) agar medium with rolled oat flakes (Quaker Oats
58 Company®) in Petri dishes (90mm Ø). They were kept in the dark in a thermoregulated chamber at a
59 temperature of 20 degrees Celsius and a humidity of 80% from Monday to Friday. They were
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1 transferred every day on a new agar medium on which was spread a single layer of rolled oat flakes.
2 During the weekends, slime moulds were unfed and kept at 12°C. One day before each experiment, all
3 plasmodia were transferred on a food medium consisting of finely ground oat flakes (5% w/v) mixed
4 with 1% agar gel, hereafter referred as “oat gel”.
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8 9 **(b) Migration speed on various substrates as a function of slime moulds age**

10 The aim of this experiment was to investigate how age affects the slime mould migration speed on
11 various substrates (Figure 1a). Six groups of 10 large plasmodia of different ages (1, 17, 32, 54, 74
12 and 94 weeks old) were used to conduct this experiment. Circular samples (10 mm Ø) were cut from
13 each plasmodium and gently placed in contact with an oat gel bridge (35 mm long, 10 mm wide) in an
14 experimental arena (Petri dish 12mm*12mm). The bridge either contained an aversive substance: NaCl
15 (100mM, 0.6% w/v) (Oat + NaCl bridge) or NaNO₃ (100mM, 0.8% w/v) (Oat + NaNO₃ bridge) or not
16 (Oat). Experimental arenas housed 8 bridges and were kept in a thermoregulated chamber at a
17 temperature of 25 degrees Celsius. After about 8 hours of incubation, the distance travelled by the
18 slime moulds on each bridge was measured with a ruler. We computed the migration speed as the
19 distance travelled divided by the time spent in contact with the bridge. A minimum of 48 samples were
20 tested for each substrate and each age group, leading to total of 2396 assays.
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31 **(c) Migration speed at different temperatures as a function of slime moulds** 32 **age**

33 Slime moulds have been shown to suffer both from low and high temperature which affect the shuttle
34 streaming and therefore locomotion [72]. The aim of this experiment was to investigate how age affects
35 the slime mould migration speed under different temperatures (Figure 1b). Five groups of 10 large
36 plasmodia of different ages (8, 24, 61, 81 and 101 weeks old) were used to conduct this experiment.
37 Circular samples (10 mm Ø) were cut from each plasmodium and gently placed in contact with an oat
38 gel bridge (35 mm long, 10 mm wide) in an experimental arena (Petri dish 12mm*12mm).
39 Experimental arenas housed 8 bridges and were kept in thermoregulated chambers at a temperature of
40 10, 25 or 38.5°C degrees Celsius. 25°C is a standard temperature to rear *Physarum polycephalum* while
41 10°C and 38.5°C represents extremes conditions. After about 8 hours of incubation, the distance
42 travelled by the slime moulds on each bridge was measured with a ruler. We computed the migration
43 speed as the distance travelled divided by the time spent in contact with the bridge. Numerous slime
44 moulds did not move at all during the experiments at extreme temperatures (10 or 38.5°C), they were
45 not taken into account to compute speed but we calculated the proportion of motionless slime moulds
46 for each age group and each temperature. A minimum of 80 samples were tested for each temperature
47 and each age group, leading to total of 3533 assays.
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(d) Decision making ability as a function of slime moulds age

Slime moulds have been shown to succeed in selecting the best option when offered multiple alternatives [22,27,45,73]. To investigate how age affects slime mould ability to make a decision, we studied how a slime mould distributed itself between two agar gel bridges containing or not an aversive substance (NaCl or NaNO₃) (Figure 1c). Under non aversive condition, the slime moulds were offered a choice between a high-quality oat gel (5% w/v) and a low-quality one (2.5% w/v). Under aversive condition, the slime moulds were given a choice between a high-aversive option (0.6% w/v NaCl or NaNO₃) and a low-aversive option (0.4% w/v NaCl or NaNO₃). Five groups of 10 large plasmodia of different ages (6, 22, 59, 79 and 99 weeks old) were used to conduct this experiment. Circular samples (10 mm Ø) were cut from each plasmodium, placed between two bridges (35 mm long, 10 mm wide) in experimental arenas (Petri dish 12mm*12mm). Experimental arenas housed 4 binary choices and were kept in a thermoregulated chamber at a temperature of 25 degrees Celsius. After about 8 hours of incubation, the distance travelled (in mm) on each bridge was measured with a ruler. We computed a decision-making performance as the distance covered on the best bridge (high quality or low aversive substrate) divided by the total distance. A minimum of 235 samples were tested for each age and each binary choice, leading to total of 4206 assays

(e) Learning ability as a function of slime moulds age

Slime moulds have been shown to be capable of habituation, a simple form of learning, when repeatedly exposed to an innocuous aversive substance [40,74]. To investigate how age affects slime moulds' ability to learn, we studied how slime moulds habituate to an aversive substance [25,40]. A bridge crossing experiment adapted from [40] was conducted to habituate the slime mould to an aversive substance (NaCl 100mM, 0.6% w/v) (Figure 1d). We used two groups of 10 large plasmodia of two different ages 12 and 74 weeks old. Before starting the habituation experiment, to accustom the slime moulds to the experimental set-up, 10 circular samples (Ø 10 mm) were cut from each plasmodium and introduced in an experimental arena (Petri dish 12mm*12mm). The samples were then connected to an oat gel bridge (35 mm long, 10 mm wide). After a day, the slime moulds had covered the bridge, and a circular sample was cut from the bridge and transferred to new experimental arenas to start the habituation. On day 1 of the habituation, half of the samples were offered an oat gel bridge containing NaCl (substrate: Oat+NaCl, treatment: habituated, N=50 for each age group) while the remaining half had to cross a bridge without NaCl (substrate: Oat, treatment: control, N=50 for each age group). The following two days (day 2 and day 3), a circular sample was cut from each bridge daily and transferred to another arena where they were offered a new bridge. On day 4, once the habituation training was completed, we cut one or two circular samples from each bridge and transferred them to a new arena. Half of the habituated and control samples were offered a bridge containing NaCl (substrate: Oat+NaCl) while the remaining half had to cross a bridge without NaCl

(substrate: Oat). Thus, on day 4 the control group is either facing a bridge containing NaCl for the first time (N=30 for each age group) or a bridge without NaCl (N=30 for each age group) while the habituated group is either facing a bridge containing NaCl for the fourth time (N=30 for each age group) or a bridge without NaCl (N=30 for each age group). Every day, after about 8 hours of incubation, the distance travelled (in mm) on each bridge was measured with a ruler. Data collected on day 1 allowed us to confirm that slime moulds showed a clear aversive behaviour toward the NaCl while data collected on day 4 enable us to test if slime mould learn to ignore the aversive substance. On day 4 we synthesized the results with an aversion index HI and CI as in [41].

$$HI = \left| \frac{1}{N} \sum_{i=1}^N \frac{HON_i - \overline{HO}}{sd(\overline{HO})} \right| \quad \text{or} \quad CI = \left| \frac{1}{N} \sum_{i=1}^N \frac{CON_i - \overline{CO}}{sd(\overline{CO})} \right|$$

HON, CON, HO and CO are the habituated (H) and control slime moulds (C) on Oat+NaCl (ON) and Oat (O) respectively. Using those indexes allowed us to normalize each variable value corresponding to the treatments HON and CON by the mean and the standard deviation of their respective control HO and CO. Values clearly above 0 indicate an aversion towards the repellent, whereas values close to zero indicate habituation to the repellent *i.e.* slime moulds react the same way to Oat+NaCl and Oat.

(f) Recovery ability following dormancy as a function of slime moulds age

To investigate how age affects slime mould ability to recover, we studied how fast old slime moulds migrate after a dormancy period (Figure 1e). We used two groups of 10 large plasmodia of two different ages 60 and 100 weeks old, referred as “old” and “very old”. To initiate the transition from plasmodia to sclerotia, we cut 2 circular samples from each plasmodium ($\varnothing = 20$ mm), placed each of them on a moist paper filter ($\varnothing = 145$ mm) in a petri dish ($\varnothing = 145$ mm) and kept them in a thermoregulated chamber at a temperature of 30 degrees Celsius for two days to dry. The sclerotia were then stored for a week before being re-initiated to obtain new plasmodia. A week later, we then had 40 large plasmodia, 20 were one week old and originated from old and very old plasmodia (treatment: dormancy) and 20 were old and very old (treatment: no dormancy). Circular samples ($\varnothing 10$ mm) were cut from each plasmodium and gently placed in contact with an oat gel bridge (nutritive substrate) or an agar gel bridge (agar 1%w/v, non-nutritive substrate) in an experimental arena (Petri dish 12mm*12mm). Experimental arenas housed 8 bridges and were kept in a thermoregulated chamber at a temperature of 25 degrees Celsius. After about 8 hours of incubation, the distance travelled by the slime moulds on each bridge was measured with a ruler. We computed the migration speed as the distance travelled divided by the time spent in contact with the bridge. A minimum of 80 samples were tested for each age group (old, very old) and each treatment (dormancy or not), leading to total of 640 assays.

(g) Fusion as a function of slime moulds age

Fusion constitutes a defining feature of the lifestyle of slime moulds. To investigate how age affects slime mould ability to fuse, we studied how fast two slime moulds fuse as function of their respective age (Figure 1f). We used two groups of 10 large plasmodia of two different ages 18 and 75 weeks old, referred as “young” and “old”. Circular samples (10 mm Ø) were cut from each plasmodium and gently placed in contact with an agar gel bridge (15 mm long, 15 mm wide) in an experimental arena (Petri dish 12mm*12mm). We placed a sample at each extremity of the bridge and tested the following pairs: young/young (N=220), young/old (N=330) and old/old (N=220). Experimental arenas housed 8 pairs of slime moulds. They were kept in a thermoregulated dark room at a temperature of 21 degrees Celsius. We conducted behavioural scan observations using a flash light to record the latency to membrane and vein fusion. Membranes were considered as fused when we could not see the delimitation between the two slime moulds while veins were considered as fused when we could discern a vein going from one slime mould to the other. The latency between two scans was less than 5 minutes. Pairs that did not fuse after 9h or that fused with their neighbouring slime moulds in the arenas were removed from the analysis (young/young: 26 out of 220, young/old: 25 out of 330 and old/old: 18 out of 220)

(h) Recovery ability following fusion as a function of slime moulds age

Slime mould fusion could provide the potential for cell recovery through resource sharing. Recently, it was shown that a learned behaviour can be transferred during fusion [25]. In this experiment, we measure migration speed after fusion as a function of slime moulds age (Figure 1g). We used two groups of 10 large plasmodia of two different ages 5 and 54 weeks old, referred as “young” and “old”. Circular samples (10 mm Ø) were cut from each plasmodium and gently placed by pairs in an experimental arena (Petri dish 12mm*12mm). Each experimental arenas housed 4 pairs. We tested the following pairs: young/young (N=100), young/old (N=100) and old/old (N=100). Fusion was allowed by bringing the slime moulds into contact for 3 hours (see Vogel and Dussutour 2016). After the fusion, the slime moulds were gently separated at the point of contact using a spatula. Then, all slime moulds were required to cross an oat gel bridge (35 mm long, 10 mm wide). Experimental arenas housed 8 bridges and were kept in a thermoregulated chamber at a temperature of 25 degrees Celsius. After about 8 hours of incubation, the distance travelled by the slime moulds on each bridge was measured with a ruler.

(i) Detection of conspecifics as a function of slime moulds age

Slime moulds can sense and respond to chemical cues released in the environment by conspecifics in a foraging context [21,44]. They release calcium while foraging which is attractive to other slime moulds [21]. In this experiment we monitored the directional movement response evoked in slime

1 moulds in the presence of substrates previously explored by clone mates of different age (Figure 1h).
2 We used two groups of 10 large plasmodia of two different ages 3 and 52 weeks old, referred as
3 “young” and “old”. Circular Petri dishes (diameter, $\text{\O} = 90$ mm) containing a layer of 1% w/v agar gel
4 were used as experimental arenas. Once the agar in the Petri dish had set, three holes were punched (\O
5 = 10 mm, inter- distance = 20 mm). One hole was filled with a young and an old slime mould ($\text{\O} = 10$
6 mm) sitting on 5% w/v oat gel. The other two holes were filled with experimental substrates ($\text{\O} = 10$
7 mm) which consisted in agar gel explored for 24 h by well-fed young and old slime mould. To obtain
8 the experimental substrates, a slime mould was allowed to cover completely an agar gel while being
9 fed with oat flakes (making sure that the food was never in contact with the agar substrate, see [21] for
10 details). Then we removed the slime mould, and rinsed the agar substrate with distilled water just before
11 the experiment. The slime mould would typically explore its environment by expanding in all
12 directions for a short distance to finally migrate in a specific direction, eventually contacting one
13 experimental substrate. The experimental substrate that was reached first was taken to imply a positive
14 response (*i.e.*, a relative preference for the cues enclosed in the experimental substrate over the
15 alternative). For each assay, we recorded which substrate was contacted first and the latency to reach
16 each substrate. We replicated each binary choice 300 times with old and young slime moulds.
17 Experiments were recorded using digital cameras (EOS 70D, Canon) which took a picture every 5 min
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31 (j) Statistical analyses

32 All statistics were performed with RStudio (Version 1.2.1335). To assess the difference in the various
33 parameters measured between the treatments, we used linear mixed models (LMM) or generalised
34 mixed models (GLMM) (function lmer or glmer). The package lme4 [75] was used for all mixed
35 models. The lmerTest and Car packages were then used to run Type III analysis of variance on the
36 calculated LMMs and GLMMs, extracting p-values for F-tests and Wald-tests. Assumptions for all
37 LMMs were checked using standard procedure: diagnostic of quantile-quantile normal plots and
38 Shapiro-Wilk test. The models were fitted by specifying the fixed effects (explanatory variables)
39 depending on the experiment and a random effect: the plasmodium identity. The dependent variables
40 that did not fit linear model requirements were transformed using the “bestNormalize” function
41 (“bestNormalize” package). Continuous variables were centred and scaled if needed. The outcomes of
42 all the models are presented in the supplementary information (Table S1-S13)
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54 Results

55 (a) Migration speed on various substrates as a function of slime moulds age

56 In the first experiment, we investigated if slime moulds migration speed is affected by age on different
57 substrates. Slime moulds migrated faster on plain oat gel than on oat gels containing an aversive
58 substance (LMM, substrate $F=67.01$, $p<0.001$, table S1, figure 2). For all substrates, migration speed
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1 decreased as age increased (LMM, age $F=80.52$, $p<0.001$, table S1, figure 2). On plain oat gel,
2 migration speed declined drastically until the slime moulds were 30 weeks old then decreased gently.
3 On oat gel containing NaNO_3 , migration speed reached a plateau when the slime moulds were over 30
4 weeks old. On oat gel containing NaCl , migration speed declined continuously with age (LMM age *
5 substrate $F=28.08$, $p<0.001$, table S1, figure 2). Hence, NaNO_3 was the most aversive substance for
6 young slime moulds while for old slime moulds it was NaCl .
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11 12 **(b) Migration speed at different temperatures as a function of slime moulds** 13 **age** 14

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16 In the second experiment, we examined if slime moulds' migration speed was affected by age at
17 different temperatures. Slime moulds migrated faster at standard temperature than at cold or hot
18 temperature (GLMM, temperature $\chi^2=4724.85$ $p<0.001$, table S2, figure 3). As previously, at standard
19 temperature, migration speed declined drastically with age at the beginning then decreased gently when
20 the slime moulds were over 25 weeks old. At both low and high temperatures, the speed was the lowest
21 for young slime moulds (8 weeks old), then increased until the slime moulds reached an intermediate
22 age between 24 and 61 weeks old and finally decreased slightly with age (GLMM, age $\chi^2=5.67$
23 $p=0.017$, age * temperature $\chi^2=43.26$ $p<0.001$, table S2, figure 3). The proportion of motionless slime
24 moulds was also affected by age (GLMM, age $\chi^2=10.11$ $p<0.001$, Table S3, figure 3) and temperature
25 (GLMM, temperature $\chi^2=200.47$ $p<0.001$, table S3, figure 3). The youngest (8 weeks old) and oldest
26 slime moulds (101 weeks old) remained most often motionless at both extreme temperatures with the
27 youngest being more vulnerable to cold and the oldest to heat (GLMM, age * temperature $\chi^2=39.58$
28 $p<0.001$, table S3, figure 3).
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40 **(c) Decision making ability as a function of slime moulds age** 41

42 In the third experiment, we estimated slime moulds' ability to make a decision as a function of age and
43 substrate. As previously, slime moulds migrated faster on plain oat gel than on oat gels containing an
44 aversive substance (GLMM, substrate $\chi^2=451.80$ $p<0.001$, table S4) and for all substrates migration
45 speed decreased as age increases (GLMM, age $\chi^2=5.91$ $p<0.001$, age * substrate $\chi^2=18.88$ $p<0.001$,
46 table S4). On average, slime moulds had better performances under non aversive conditions (GLMM,
47 substrate $\chi^2=141.34$ $p<0.001$, table S5, figure 4). However, under aversive conditions, decision making
48 performance increased with age while it remained constant under non aversive conditions (GLMM,
49 age $\chi^2=4.22$ $p=0.040$, age * substrate $\chi^2=111.82$ $p<0.001$, table S5, figure 4). Hence, young slime mould
50 had better performances under non aversive condition while it was the contrary for old slime moulds.
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59 **(d) Learning ability as a function of slime moulds age** 60

In the fourth experiment, we investigated if slime moulds' ability to habituate to an aversive substance
depended on age. On day 1, slime moulds migrated faster on plain oat gel than on oat gels containing

1 NaCl (LMM, substrate $F=75.86$ $p<0.001$, table S6, figure 5a) and migration speed was higher for
2 young than for old slime moulds (LMM, age $F=15.27$ $p=0.001$, age * substrate $F=3.76$ $p=0.054$, table
3 S6, figure 5a). On day 4, habituated and control slime moulds were tested for habituation and were
4 required to cross a bridge containing NaCl. Control slime moulds showed a strong aversive behaviour
5 and a high aversion index. In contrast, habituated slime moulds, encountering NaCl for the fourth time,
6 showed no aversive behaviour and an aversion index close to zero (LMM, treatment $F=23.54$ $p<0.001$,
7 table S7, figure 5b). Hence, they learned to ignore the aversive substance. Slime moulds age did not
8 affect their learning performance (LMM, age $F=2.15$ $p=0.145$, age*treatment $F=1.92$ $p=0.169$, table
9 S7, figure 5b)

20 **(e) Recovery ability as a function of slime moulds age**

21 In the fifth experiment, we tested if slime moulds recovered their original migration speed after a
22 dormancy period. Slime moulds migrated faster on oat gel than on agar gels (LMM, substrate $F=30.10$
23 $p<0.001$, table S8, figure 6) regardless of age and treatment (LMM, substrate * age $F=0.11$ $p=0.741$,
24 substrate * treatment $F=2.54$ $p=0.111$, table S8, figure 6). Slime moulds that were turned into a dormant
25 state and reinitiated were significantly faster than their counterparts that remained as plasmodia (LMM,
26 treatment $F=59.52$ $p<0.001$, table S8, figure 6). Age of the dormant slime mould had no effect on speed
27 following dormancy (treatment * age, $F=0.09$ $p=0.772$, table S8, figure 6).

34 **(f) Fusion as a function of slime moulds age**

35 In the sixth experiment we examined if the fusion process was affected by age. The latency for the
36 membrane to fuse was shorter for pairs of young slime moulds or mixed pair (young/old) than for pairs
37 of old ones (LMM, age $F=3.63$ $p=0.034$ table S9, figure 7a). Vein fusion, following membrane fusion,
38 also occurred earlier in pair of young slime moulds than in the other pairs (LMM, age $F=4.21$ $p=0.030$,
39 table S10, figure 7b).

46 **(g) Recovery ability following fusion as a function of slime moulds age**

47 In the fifth experiment, we tested if old slime moulds recovered their original migration speed after
48 fusing with young ones. Young slime moulds were faster than old slime mould when they were allowed
49 to fuse with a slime mould of the same age (LMM, age $F=19.68.52$ $p<0.001$, table S11, figure 8).
50 However, old slime moulds, that were allowed to fuse with a young one, were significantly faster than
51 their counterparts that fused with an old one (LMM, age*pair type $F=33.84$ $p<0.001$, table S11, figure
52 8). On the contrary young ones that were allowed to fuse with an old slime mould were slower than
53 their counterparts that fused with a young one.

(h) Detection of conspecifics as a function of slime moulds age

In the last experiment, we investigated if slime moulds could perceive a difference in cues left by slime moulds of different ages. As previously shown, young slime moulds were faster than old ones (GLMM, age $\chi^2=14.44$ $p<0.001$, table S12, figure 9). Both young and old slime moulds contacted first the substrate explored by a young slime mould significantly more often than the substrate explored by an old one (GLMM, observed vs expected: $\chi^2=5.67$ $p=0.017$, table S13, figure 9).

Discussion

In many organisms, ageing is accompanied by deficits in behavioural performance. In this study, we were able to maintain a culture of slime moulds for more than two years and track their behaviour throughout time. We revealed that old slime moulds move slower than young ones on neutral, nutritive and aversive substrates in all our experiments. We confirmed that aversive substrates slowed down slime moulds regardless of age [25,41,76,77]. Sodium is known to decrease migration rate in slime moulds via a depolarization of the membrane potential [78]. An interesting observation was that sodium chloride had a stronger effect than sodium nitrate on old slime moulds while it was the opposite for young slime moulds. In many eukaryotic cells, high extracellular NaCl increases reactive oxygen species (ROS), causes DNA damage and promotes senescence [79,80]. Given that old slime moulds already suffered from molecular damages due to senescence (effects listed in the introduction [62–64,68–70]), NaCl might have amplified those damages. Interestingly, old slime moulds were better at avoiding high concentration of NaCl or NaNO₃ than young slime moulds, suggesting that they were indeed more susceptible to chemical stressors and actively avoided them. As a matter of fact, we did not observe any significant differences in decision abilities when slime moulds were facing two food sources of different quality.

We then revealed that migration speed depended on age under extreme temperature. Temperature is known to be one of the main factors affecting the shuttle streaming in slime moulds. The contraction period decreases from 13min at 1°C to 1.26 min at 30°C and then increases past 32°C [57,58,81,82]. At 42°C, the shuttle streaming is totally arrested and the membrane exhibits alterations [81]. In our experiment, all slime moulds were slowed down by extreme temperatures and this effect increased with age when slime moulds age ranged from 25 to 100 weeks. Temperature is known to contribute to senescence in most living-organisms [83], including slime moulds [71]. Susceptibility to thermal stress increases as an organism ages due to more molecular damage being generated and accumulated over time [84]. Yet, surprisingly, the effect of temperature was the strongest for the youngest slime moulds which were 8 weeks old. As our culture alternated between 25°C during the week and 12°C during the weekend, it might be possible that a thermotolerance was indirectly induced over time. Signs of habituation to low temperature has already been observed in slime moulds [85]. In *P. polycephalum* as in most living organisms, expression of heat-shock protein (HSP) increases after

1 exposure to a moderate thermal stress [86]. HSPs function as molecular chaperones, they reduce
2 molecular damage induced by extreme temperatures allowing the organism to cope with this stressor
3 [87–90]. Usually, how much HSPs an organism produces correlates with the level of stress to which it
4 is exposed. Therefore, in our experiment, we can presume that the level of expression of HSPs was
5 higher in older slime moulds than in younger ones as they experienced more periodic changes in
6 temperature over time. Thus, older slime moulds might have been more susceptible to thermal stress
7 owing to their age but also more thermotolerant due to their past experiences than younger slime
8 moulds.
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10 Recently [74,91], it was revealed that slime moulds are capable of habituation, a simple form
11 of learning, when they are repeatedly exposed to a chemical stimulus. In animals, learning performance
12 displays a progressive decrease with age. However when it comes to habituation, it was observed that
13 older organisms habituate more rapidly than young ones in *Aplysia californica* [92] and *Caenorhabditis*
14 *elegans* [93] or as fast as young ones in *Drosophila melanogaster* [94], mice [95] and rats [96]. Here,
15 we showed that, habituation performance is preserved in old slime moulds as well. In animals, it was
16 shown that the response threshold to the stimulus was higher in old animals than in young ones which
17 could explain their performance [92,95]. In contrast, in our experiment, we showed that the aversion
18 level toward NaCl did not vary with age. On the contrary, in our first experiment, older individuals
19 seemed more susceptible to NaCl than younger ones. Habituation to NaCl in slime moulds relies, in
20 part, on NaCl uptake [41]. In plants, yeast and cellular slime moulds, sodium is driven into the cell
21 passively through non selective cationic channels [97–99]. Therefore, it is possible that this passive
22 transport of sodium throughout the membrane is not affected by age.
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24 In slime moulds, sclerotia have been found to survive under adverse environmental conditions
25 for long periods up to 3 years. In the slime mould *Didymimim iridis*, a related species, the aging process
26 stops when the plasmodium differentiates into a sclerotium [100]. Once the sclerotium is revived, the
27 plasmodium then lives the remainder of its characteristic life spans [100]. In contrast in this study, we
28 observed a recovery phenomenon when aged slime moulds went throughout a dormant stage. After
29 dormancy, old slime moulds migrated as fast as young slime moulds and faster than old slime moulds
30 which had remained in a plasmodial stage. Past works have shown that oxidative stress defences are
31 involved in the transition from plasmodium to sclerotium in *P. polycephalum* [101,102]. However,
32 cell senescence is in part due to oxidative damage to DNA, RNA and proteins by mitochondrial reactive
33 oxygen species (ROS) which accumulate with age [103]. Hence, one hypothesis might be that oxidative
34 stress defences expressed during differentiation were responsible for the elimination of harmful ROS
35 and, as a consequence, the behavioural recovery observed in old slime molds after dormancy.
36 However, we only recorded the behaviour of the slime moulds one week after they were reinitiated
37 from sclerotia and we cannot rule out that this rejuvenation phenomenon was only transient and did
38 not affect the plasmodium lifespan.
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1 In slime moulds, cell fusion is extremely common. In a related species *Didymium nigripes*, it
2 was shown that slime moulds lose the ability to fuse over time [104]. In this study, in contrast, we
3 showed that fusion occurred regardless of slime mould age. As old slime moulds were slower than their
4 younger counterparts, their latency to fuse was simply longer. Vein fusion took also a longer time in
5 old slime moulds than in young ones, but fusion was never aborted. We also revealed that an old slime
6 mould could recover its migration speed performance after fusing with a young counterpart. Resource
7 sharing between cells is often observed as a strategy to cope with environmental stress. For instance,
8 cells can exchange learned information [25], mitochondria [105] or membrane material [106] via cell
9 fusion. It is well known that cells accumulate DNA, protein and metabolite damage over time [107]. In
10 young cells, most of the damages are cleared by maintenance processes [107]. However, as not all
11 damages can be repaired, irreparable damage rises in the cell through time [107]. In aged cells, the
12 maintenance systems can be damaged themselves, amplifying the burden of damages [107]. In our
13 experiment, it might be possible that the damages accumulated over time in an old slime mould were
14 cleaned or repaired by the maintenance system of its younger counterpart. Interestingly, in a related
15 species, *Didymium iridis*, when age-hybrid slime moulds were formed by the fusion of young and old
16 individuals, 58 % of the new slime moulds acquired the remaining life span of the older individuals,
17 22 % died concurrently with the younger individuals and 20% died at an intermediate age [108]. The
18 authors concluded that it is the oldest slime mould that determines the lifespan of the age-hybrid slime
19 mould [100,109]. In our experiment, we did not follow the age-hybrid slime moulds behaviour over
20 time. Hence, even if we observed a form of recovery process in old slime moulds after fusion, we
21 cannot rule out that they would have died concurrently with slime moulds of the same age in absence of
22 fusion.

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39 As *P. polycephalum* migrates on the substrate, it leaves behind an extracellular slime [42,110].
40 Previous works have shown that previously explored substrate might be repulsive or attractive
41 depending on the physiological status of the slime mould that explored the substrate [21,44]. A
42 substrate explored by a starved, irradiated or poisoned slime mould is actively avoided [44] while a
43 substrate explored by a well-fed slime mould is attractive [21]. Calcium has been identified as the main
44 chemical that mediates attraction [21] whereas the substances responsible for the aversion remain to
45 be identified. In this study, we showed that slime moulds were able to use age-related cues released by
46 conspecifics and make a decision accordingly. We revealed that slime moulds, regardless of their age,
47 were more attracted by cues released by younger slime moulds than by older ones. As we did not notice
48 any fusion delay when a young slime mould was facing an old one in the fusion experiment, we believe
49 that the substrate explored by an old slime mould was not aversive but only less attractive. The
50 information supplied by these excretions would appear to be crucial for both young and old slime
51 mould. A young slime mould would benefit from avoiding fusion with an aged slime mould as it could
52 inherit its damages. Indeed, we noticed that after fusing with an old counterpart, a young slime mould

1 was somewhat slower. In contrast, an old slime mould would gain from fusing with a young slime
2 mould, as it would allow it to recover. In many animals from flies to humans [111–115], as an organism
3 ages, the effect of senescence on both the perception and processing of information might affect their
4 response to the presence of others. Here, we did not observe any effect of age on social interactions.
5 since older individuals were still able to fuse with others (although in a slower fashion) and detect
6 conspecifics.
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11 Although many studies have studied behaviour in single cell organisms, few have taken the
12 step of looking for changes in behaviour over the lifetime of individuals. This study extends our
13 knowledge of the behavioural plasticity of unicellular organisms and establish acellular slime molds as
14 a promising model to investigate the effect of aging on behaviour at the cellular level. Their ability to
15 fuse with congeners and their peculiar lifecycle, might help us to understand how cellular metabolic
16 activity and cellular defences are regulated and coordinated, and how a cell can counteract the effects
17 of ageing.
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27 References

- 28 1. Waters CM, Bassler BL. 2005 Quorum sensing: cell-to-cell communication in bacteria. *Annu. Rev. Cell Dev. Biol.* **21**, 319–346. (doi:10.1146/annurev.cellbio.21.012704.131001)
- 29 2. Whiteley M, Diggle SP, Greenberg EP. 2017 Progress in and promise of bacterial quorum sensing
30 research. *Nature* **551**, 313–320. (doi:10.1038/nature24624)
- 31 3. Gross JD, Peacey MJ, Trevan DJ. 1976 Signal emission and signal propagation during early
32 aggregation in *Dictyostelium discoideum*. *J. Cell Sci.* **22**, 645–656.
- 33 4. Bonner JT. 1959 „The cellular Slime moulds” Princeton Univ. Press. New Jersey)
- 34 5. Sawai S, Thomason PA, Cox EC. 2005 An autoregulatory circuit for long-range self-organization in
35 *Dictyostelium* cell populations. *Nature* **433**, 323–326.
- 36 6. Gregor T, Fujimoto K, Masaki N, Sawai S. 2010 The onset of collective behavior in social amoebae.
37 *Science (80-.).* **328**, 1021–1025.
- 38 7. Bell G. 1984 Evolutionary and nonevolutionary theories of senescence. *Am. Nat.* **124**, 600–603.
- 39 8. Kirkwood TBL, Austad SN. 2000 Why do we age? *Nature* **408**, 233–238.
- 40 9. Moger-Reischer RZ, Lennon JT. 2019 Microbial ageing and longevity. *Nat. Rev. Microbiol.* **17**, 679–
41 690. (doi:10.1038/s41579-019-0253-y)
- 42 10. Ackermann M, Chao L, Bergstrom CT, Doebeli M. 2007 On the evolutionary origin of aging. *Aging Cell* **6**, 235–244.
- 43 11. Łapińska U, Glover G, Capilla-Lasheras P, Young AJ, Pagliara S. 2019 Bacterial ageing in the
44 absence of external stressors. *Philos. Trans. R. Soc. B* **374**, 20180442.
- 45 12. Florea M. 2017 Aging and immortality in unicellular species. *Mech. Ageing Dev.* **167**, 5–15.
46 (doi:https://doi.org/10.1016/j.mad.2017.08.006)

- 1 13. Crane MM, Kaeberlein M. 2018 The paths of mortality: How understanding the biology of aging
2 can help explain systems behavior of single cells. *Curr. Opin. Syst. Biol.* **8**, 25–31.
3 (doi:https://doi.org/10.1016/j.coisb.2017.11.010)
4
- 5 14. Reid CR, Latty T. 2016 Collective behaviour and swarm intelligence in slime moulds. *FEMS*
6 *Microbiol. Rev.* , fuw033. (doi:10.1093/femsre/fuw033)
7
- 8 15. Takamatsu A, Fujii T, Endo I. 2000 Time delay effect in a living coupled oscillator system with the
9 plasmodium of *Physarum polycephalum*. *Phys. Rev. Lett.* **85**, 2026.
10
- 11 16. Boussard A, Fessel A, Oettmeier C, Briard L, Döbereiner H-G, Dussutour A. 2020 Adaptive
12 behavior and learning in slime moulds: the role of oscillations. *Philos. Trans. R. Soc. London. B*
13 **376**, 20190757.
14
- 15 17. Yoshiyama S, Ishigami M, Nakamura A, Kohama K. 2010 Calcium wave for cytoplasmic streaming
16 of *Physarum polycephalum*. *Cell Biol. Int.* **34**, 35–40.
17
- 18 18. Alim K, Amselem G, Peaudecerf F, Brenner MP, Pringle A. 2013 Random network peristalsis in
19 *Physarum polycephalum* organizes fluid flows across an individual. *Proc. Natl. Acad. Sci.* **110**,
20 13306–13311.
21
- 22 19. Meyer B, Ansorge C, Nakagaki T. 2017 The role of noise in self-organized decision making by the
23 true slime mold *Physarum polycephalum*. *PLoS One* **12**, e0172933.
24
- 25 20. Vogel D, Dussutour A, Deneubourg J-L. 2018 Symmetry breaking and inter-clonal behavioural
26 variability in a slime mould. *Biol. Lett.* **14**, 20180504.
27
- 28 21. Vogel D, Nicolis SC, Perez-Escudero A, Nanjundiah V, Sumpter DJT, Dussutour A. 2015 Phenotypic
29 variability in unicellular organisms: from calcium signalling to social behaviour. *Proc. R. Soc. B*
30 *Biol. Sci.* **282**, 20152322.
31
- 32 22. Dussutour A, Ma Q, Sumpter D. 2019 Phenotypic variability predicts decision accuracy in
33 unicellular organisms. *Proc. R. Soc. B* **286**, 20182825.
34
- 35 23. Zabzina N, Dussutour A, Mann RP, Sumpter DJT, Nicolis SC. 2014 Symmetry restoring bifurcation
36 in collective decision-making. *PLoS Comput. Biol.* **10**, e1003960.
37
- 38 24. Fessel A, Oettmeier C, Bernitt E, Gauthier NC, Döbereiner H-G. 2012 *Physarum polycephalum*
39 Percolation as a Paradigm for Topological Phase Transitions in Transportation Networks. *Phys.*
40 *Rev. Lett.* **109**. (doi:10.1103/PhysRevLett.109.078103)
41
- 42 25. Vogel D, Dussutour A. 2016 Direct transfer of learned behaviour via cell fusion in non-neural
43 organisms. *Proc. R. Soc. B Biol. Sci.* **283**, 20162382.
44
- 45 26. Ray SK, Valentini G, Shah P, Haque A, Reid CR, Weber GF, Garnier S. 2019 Information transfer
46 during food choice in the slime mold *Physarum polycephalum*. *Front. Ecol. Evol.* **7**, 67.
47
- 48 27. Reid CR, Garnier S, Beekman M, Latty T. 2015 Information integration and multiattribute decision
49 making in non-neuronal organisms. *Anim. Behav.* **100**, 44–50.
50 (doi:10.1016/j.anbehav.2014.11.010)
51
- 52 28. Reid CR, MacDonald H, Mann RP, Marshall JAR, Latty T, Garnier S. 2016 Decision-making without
53 a brain: how an amoeboid organism solves the two-armed bandit. *J. R. Soc. Interface* **13**,
54 20160030.
55
56
57
58
59
60

- 1 29. Beekman M, Latty T. 2015 Brainless but Multi-Headed: Decision Making by the Acellular Slime
2 Mould *Physarum polycephalum*. *J. Mol. Biol.* **427**, 3734–3743. (doi:10.1016/j.jmb.2015.07.007)
- 3 30. Nakagaki T, Kobayashi R, Nishiura Y, Ueda T. 2004 Obtaining multiple separate food sources:
4 behavioural intelligence in the *Physarum plasmodium*. *Proc. R. Soc. London. Ser. B Biol. Sci.* **271**,
5 2305–2310. (doi:10.1098/rspb.2004.2856)
- 6 31. Jones J. 2015 ECC 15 - From Pattern Formation to Material Computation. , 1–369.
- 7 32. Gao C, Liu C, Schenz D, Li X, Zhang Z, Jusup M, Wang Z, Beekman M, Nakagaki T. 2019 Does being
8 multi-headed make you better at solving problems? A survey of *Physarum*-based models and
9 computations. *Phys. Life Rev.* **29**, 1–26.
- 10 33. Nakagaki T, Yamada H, Tóth Á. 2000 Maze-solving by an amoeboid organism. *Nature* **407**, 470.
- 11 34. Tero A, Takagi S, Saigusa T, Ito K, Bebbler DP, Fricker MD, Yumiki K, Kobayashi R, Nakagaki T.
12 2010 Rules for biologically inspired adaptive network design. *Science (80-.)*. **327**, 439–442.
- 13 35. Oettmeier C, Nakagaki T, Döbereiner H-G. 2020 Slime mold on the rise: The physics of *Physarum*
14 *polycephalum*. *J. Phys. D. Appl. Phys.* **53**, 310201.
- 15 36. Awad A, Pang W, Lusseau D, Coghill GM. 2021 A Survey on *Physarum Polycephalum* Intelligent
16 Foraging Behaviour and Bio-Inspired Applications.
- 17 37. Oettmeier C, Brix K, Döbereiner H-G. 2017 *Physarum polycephalum*-a new take on a classic
18 model system. *J. Phys. D. Appl. Phys.* **50**, 1–12.
- 19 38. Reid CR, Beekman M. 2013 Solving the Towers of Hanoi--how an amoeboid organism efficiently
20 constructs transport networks. *J. Exp. Biol.* **216**, 1546–1551.
- 21 39. Saigusa T, Tero A, Nakagaki T, Kuramoto Y. 2008 Amoebae Anticipate Periodic Events. *Phys. Rev.*
22 *Lett.* **100**. (doi:10.1103/PhysRevLett.100.018101)
- 23 40. Boisseau RP, Vogel D, Dussutour A. 2016 Habituation in non-neural organisms: evidence from
24 slime moulds. *Proc. R. Soc. B Biol. Sci.* **283**, 20160446. (doi:10.1073/pnas.78.8.5245)
- 25 41. Boussard A, Delescluse J, Pérez-Escudero A, Dussutour A. 2019 Memory inception and
26 preservation in slime moulds: the quest for a common mechanism. *Philos. Trans. R. Soc. B* **374**,
27 20180368.
- 28 42. Reid CR, Latty T, Dussutour A, Beekman M. 2012 Slime mold uses an externalized spatial
29 “memory” to navigate in complex environments. *Proc. Natl. Acad. Sci.* **109**, 17490–17494.
- 30 43. Kramar M, Alim K. 2021 Encoding memory in tube diameter hierarchy of living flow network.
31 *Proc. Natl. Acad. Sci.* **118**, e2007815118.
- 32 44. Briard L, Gourjade C, Bousquet C, Dussutour A. 2020 Stress signalling in acellular slime moulds
33 and its detection by conspecifics. *Philos. Trans. R. Soc. B* **375**, 20190470.
- 34 45. Dussutour A, Latty T, Beekman M, Simpson SJ. 2010 Amoeboid organism solves complex
35 nutritional challenges. *Proc. Natl. Acad. Sci.* **107**, 4607–4611. (doi:10.1073/pnas.0912198107)
- 36 46. Murugan NJ *et al.* 2021 Mechanosensation Mediates Long-Range Spatial Decision-Making in an
37 Aneural Organism. *Adv. Mater.* **33**. (doi:10.1002/adma.202008161)
- 38 47. Reid CR, Garnier S, Beekman M, Latty T. 2015 Information integration and multiattribute decision
39 making in non-neuronal organisms. *Anim. Behav.* **100**, 44–50.

- 1 48. Baumgarten W, Hauser MJB. 2013 Functional organization of the vascular network of *Physarum*
2 polycephalum. *Phys. Biol.* **10**. (doi:10.1088/1478-3975/10/2/026003)
- 3
- 4 49. Matsumoto K, Takagi S, Nakagaki T. 2008 Locomotive mechanism of *Physarum* plasmodia based
5 on spatiotemporal analysis of protoplasmic streaming. *Biophys. J.* **94**, 2492–2504.
- 6
- 7 50. Lewis OL, Zhang S, Guy RD, Del Alamo JC. 2015 Coordination of contractility, adhesion and flow in
8 migrating *Physarum* amoebae. *J. R. Soc. Interface* **12**, 20141359.
- 9
- 10 51. Baumgarten W, Hauser MJB. 2014 Dynamics of frontal extension of an amoeboid cell. *EPL*
11 (*Europhysics Lett.* **108**, 50010. (doi:10.1209/0295-5075/108/50010)
- 12
- 13 52. Durham AC, Ridgway EB. 1976 Control of chemotaxis in *Physarum* polycephalum. *J. Cell Biol.* **69**,
14 218–223.
- 15
- 16 53. Miyake Y, Tada H, Yano M, Shimizu H. 1994 Relationship between intracellular period
17 modulation and external environment change in *Physarum* plasmodium. *Cell Struct. Funct.* **19**,
18 363–370.
- 19
- 20 54. Matsumoto K, Ueda T, Kobatake Y. 1986 Propagation of phase wave in relation to tactic
21 responses by the plasmodium of *Physarum* polycephalum. *J. Theor. Biol.* **122**, 339–345.
- 22
- 23 55. Marwan W. 2001 Chapter 20 Photomovement and photomorphogenesis in *Physarum*
24 polycephalum: targeting of cytoskeleton and gene expression by light. *Compr. Ser. Photosciences*
25 **1**, 561–587. (doi:10.1016/S1568-461X(01)80024-7)
- 26
- 27 56. Wolf R, Niemuth J, Sauer H. 1997 Thermotaxis and protoplasmic oscillations in *Physarum*
28 plasmodia analysed in a novel device generating stable linear temperature gradients.
29 *Protoplasma* **197**, 121–131.
- 30
- 31 57. Wohlfarth-Bottermann KE. 1977 Oscillating contractions in protoplasmic strands of *Physarum*:
32 simultaneous tensiometry of longitudinal and radial rhythms, periodicity analysis and
33 temperature dependence. *J. Exp. Biol.* **67**, 49–59. (doi:10.1242/jeb.67.1.49)
- 34
- 35 58. Halvorsrud R, Laane MM, Giaever I. 1995 A Novel Electrical Method to Study Plasmodial
36 Contractions in *Physarum*. Synchrony and Temperature Dependence. *Biol. Rhythm Res.* **26**, 316–
37 330. (doi:10.1080/09291019509360345)
- 38
- 39 59. Block I, Wolke A, Briegleb W. 1994 Gravitational response of the slime mold *Physarum*. *Adv. Sp.*
40 *Res.* **14**, 21–34.
- 41
- 42 60. Aldrich H. 2012 *Cell biology of Physarum and Didymium V1: organisms, nucleus, and cell cycle*.
43 Elsevier.
- 44
- 45 61. Rojas C, Stephenson SL. 2021 *Myxomycetes: biology, systematics, biogeography and ecology*.
46 Academic Press.
- 47
- 48 62. Poulter RTM. 1969 Senescence in the Myxomycete *Physarum* polycephalum.
- 49
- 50 63. Nakagawa CC, Jones EP, Miller DL. 1998 Mitochondrial DNA rearrangements associated with mF
51 plasmid integration and plasmodial longevity in *Physarum* polycephalum. *Curr. Genet.* **33**, 178–
52 187. (doi:10.1007/s002940050325)
- 53
- 54 64. Hu FS, Clark J, Lott T. 1985 Recurrent senescence in axenic cultures of *Physarum* polycephalum. *J.*
55 *Gen. Microbiol.* **131**, 811–815. (doi:10.1099/00221287-131-4-811)
- 56
- 57
- 58
- 59
- 60

- 1 65. Lott T, Clark J. 1980 Plasmodial senescence in the acellular slime mold *Didymium iridis*. *Exp. Cell*
2 *Res.* **128**, 455–457. (doi:10.1016/0014-4827(80)90080-4)
- 3 66. Adamatzky A. 2017 Thirty seven things to do with live slime mould. In *Advances in*
4 *Unconventional Computing*, pp. 709–738. Springer.
- 5 67. Keller HW, Everhart SE. 2010 Importance of Myxomycetes in Biological Research and Teaching.
6 *Society* **3**, 13–27.
- 7 68. Abe T, Takano H, Sasaki N, Mori K, Kawano S. 2000 In vitro DNA fragmentation of mitochondrial
8 DNA caused by single-stranded breakage related to macroplasmodial senescence of the true
9 slime mold, *Physarum polycephalum*. *Curr. Genet.* **37**, 125–135. (doi:10.1007/s002940050019)
- 10 69. Adler PN, Holt CE. 1974 Change in properties of *Physarum polycephalum* amoebae during
11 extended culture. *J. Bacteriol.* **120**, 532–533. (doi:10.1128/jb.120.1.532-533.1974)
- 12 70. MCCULLOUGH CHR, Cooke DJ, Foxon JL, SUDBERY PE, GRANT WD. 1973 Nuclear DNA content
13 and senescence in *Physarum polycephalum*. *Nat. New Biol.* **245**, 263–265.
- 14 71. Clark J, Lott T. 1981 Aging in the acellular slime mold *Didymium iridis*: Temperature and
15 nutritional effects. *Exp. Mycol.* **5**, 369–372. (doi:10.1016/0147-5975(81)90043-8)
- 16 72. Matsumoto K, Ueda T, Kobatake Y. 1988 Reversal of thermotaxis with oscillatory stimulation in
17 the plasmodium of *Physarum polycephalum*. *J. Theor. Biol.* **131**, 175–182.
- 18 73. Latty T, Beekman M. 2010 Food quality and the risk of light exposure affect patch-choice
19 decisions in the slime mold *Physarum polycephalum*. *Ecology* **91**, 22–27.
- 20 74. Dussutour A. 2021 Learning in single cell organisms. *Biochem. Biophys. Res. Commun.* **564**, 92–
21 102. (doi:10.1016/j.bbrc.2021.02.018)
- 22 75. Bates D, Sarkar D, Bates MD, Matrix L. 2007 The lme4 package. *R Packag. version* **2**, 74.
- 23 76. Patino-Ramirez F, Boussard A, Arson C, Dussutour A. 2019 Substrate composition directs slime
24 molds behavior. *Sci. Rep.* **9**, 1–14.
- 25 77. Denbo JR, Miller DM. 1976 Factors affecting the movement of slime mold plasmodia. *Comp.*
26 *Biochem. Physiol. -- Part A Physiol.* **55**, 5–12. (doi:10.1016/0300-9629(76)90114-6)
- 27 78. Ueda T, Terayama K, Kurihara K, Kobatake Y. 1975 Threshold phenomena in chemoreception and
28 taxis in slime mold *Physarum polycephalum*. *J. Gen. Physiol.* **65**, 223–234.
- 29 79. Dmitrieva NI, Burg MB. 2007 High NaCl promotes cellular senescence. *Cell Cycle* **6**, 3108–3113.
- 30 80. Dmitrieva NI, Cui K, Kitchaev DA, Zhao K, Burg MB. 2011 DNA double-strand breaks induced by
31 high NaCl occur predominantly in gene deserts. *Proc. Natl. Acad. Sci.* **108**, 20796–20801.
- 32 81. Lomagin AG. 1978 Repair of functional and ultrastructural alterations after thermal injury of
33 *Physarum polycephalum*. *Planta* **142**, 123–134. (doi:10.1007/BF00388203)
- 34 82. Bernstam VA, Arndt S. 1974 Effects of supraoptimal temperatures on the myxomycete *Physarum*
35 *polycephalum*. *Arch. Microbiol.* **95**, 357–363. (doi:10.1007/bf02451777)
- 36 83. Keil G, Cummings E, de Magalhaes JP. 2015 Being cool: how body temperature influences ageing
37 and longevity. *Biogerontology* **16**, 383–397.
- 38 84. Conti B. 2008 Considerations on temperature, longevity and aging. *Cell. Mol. Life Sci.* **65**, 1626–
39 1630.

- 1 85. Shirakawa T, Gunji Y-P, Miyake Y. 2011 An associative learning experiment using the
2 plasmodium of *Physarum polycephalum*. *Nano Commun. Netw.* **2**, 99–105.
- 3
4 86. Gevers M, Fracella F, Rensing L. 2006 Nuclear translocation of constitutive heat shock protein 70
5 during S phase in synchronous macroplasmodia of *Physarum polycephalum*. *FEMS Microbiol.*
6 *Lett.* **152**, 89–94. (doi:10.1111/j.1574-6968.1997.tb10413.x)
- 7
8 87. Lindquist S. 1986 The heat-shock response. *Annu. Rev. Biochem.* **55**, 1151–1191.
- 9
10 88. Kregel KC. 2002 Invited review: heat shock proteins: modifying factors in physiological stress
11 responses and acquired thermotolerance. *J. Appl. Physiol.* **92**, 2177–2186.
- 12
13 89. Sørensen JG, Kristensen TN, Loeschcke V. 2003 The evolutionary and ecological role of heat shock
14 proteins. *Ecol. Lett.* **6**, 1025–1037.
- 15
16 90. Chen B, Feder ME, Kang L. 2018 Evolution of heat-shock protein expression underlying adaptive
17 responses to environmental stress. *Mol. Ecol.* **27**, 3040–3054.
- 18
19 91. Boisseau RP, Vogel D, Dussutour A. 2016 Habituation in non-neural organisms: evidence from
20 slime moulds. *Proc. R. Soc. B Biol. Sci.* **283**, 20160446.
- 21
22 92. Rattan KS, Peretz B. 1981 Age-dependent behavioral changes and physiological changes in
23 identified neurons in *Aplysia californica*. *J. Neurobiol.* **12**, 469–478.
- 24
25 93. Beck CDO, Rankin CH. 1993 Effects of aging on habituation in the nematode *Caenorhabditis*
26 *elegans*. *Behav. Processes* **28**, 145–163.
- 27
28 94. Le Bourg E. 1983 Aging and habituation of the tarsal response in *Drosophila melanogaster*.
29 *Gerontology* **29**, 388–393.
- 30
31 95. Brennan MJ, Allen D, Aleman D, Azmitia EC, Quartermain D. 1984 Age differences in within-
32 session habituation of exploratory behavior: effects of stimulus complexity. *Behav. Neural Biol.*
33 **42**, 61–72.
- 34
35 96. Shukitt-Hale B, Casadesus G, Cantuti-Castelvetri I, Joseph JA. 2001 Effect of age on object
36 exploration, habituation, and response to spatial and nonspatial change. *Behav. Neurosci.* **115**,
37 1059.
- 38
39 97. De Hertogh B, Hancy F, Goffeau A, Baret P V. 2006 Emergence of species-specific transporters
40 during evolution of the hemiascomycete phylum. *Genetics* **172**, 771–781.
- 41
42 98. Müller U, Hartung K. 1990 Properties of three different ion channels in the plasma membrane of
43 the slime mold *Dictyostelium discoideum*. *Biochim. Biophys. Acta - Biomembr.* **1026**, 204–212.
44 (doi:https://doi.org/10.1016/0005-2736(90)90065-V)
- 45
46 99. Demidchik V, Davenport RJ, Tester M. 2002 Nonselective cation channels in plants. *Annu. Rev.*
47 *Plant Biol.* **53**, 67–107.
- 48
49 100. Lott T, Clark J. 1982 Sclerotization in Relation to Plasmodial Senescence in the Acellular Slime
50 Mould *Didymium iridis*. *Microbiology* **128**, 1483–1487.
- 51
52 101. Schreckenbach T, Werenskiold A-K. 1986 Gene expression during plasmodial differentiation. In
53 *The Molecular Biology of Physarum polycephalum*, pp. 131–150. Springer.
- 54
55 102. Allen RG, Newton RK, Sohal RS, Shipley GL, Nations C. 1985 Alterations in superoxide dismutase,
56 glutathione, and peroxides in the plasmodial slime mold *Physarum polycephalum* during
57
58
59
60

- 1 differentiation. *J. Cell. Physiol.* **125**, 413–419.
- 2
- 3 103. López-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. 2013 The hallmarks of aging. *Cell*
- 4 **153**, 1194–1217.
- 5
- 6 104. Kerr NS, Waxlax JN. 1968 A yellow variant of the eumycetozoon *Didymium nigripes* which
- 7 exhibits aging. *J. Exp. Zool.* **168**, 351–361. (doi:10.1002/jez.1401680306)
- 8
- 9 105. Spees JL, Olson SD, Whitney MJ, Prockop DJ. 2006 Mitochondrial transfer between cells can
- 10 rescue aerobic respiration. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 1283–1288.
- 11 (doi:10.1073/pnas.0510511103)
- 12
- 13 106. Vassallo CN, Wall D. 2016 Tissue repair in myxobacteria: A cooperative strategy to heal cellular
- 14 damage. *BioEssays* **38**, 306–315. (doi:10.1002/bies.201500132)
- 15
- 16 107. Gladyshev VN *et al.* 2021 Molecular damage in aging. *Nat. Aging* **1**, 1096–1106.
- 17
- 18 108. Clark J, Hakim R. 1980 Aging of plasmodial heterokaryons in *Didymium iridis*. *Mol. Gen. Genet.*
- 19 *MGG* **178**, 419–422.
- 20
- 21 109. Clark J, Lott T. 1989 Age heterokaryon studies in *Didymium iridis*. *Mycologia* **81**, 636–638.
- 22
- 23 110. Reid CR, Beekman M, Latty T, Dussutour A. 2013 Amoeboid organism uses extracellular
- 24 secretions to make smart foraging decisions. *Behav. Ecol.* **24**, 812–818.
- 25
- 26 111. Almeling L, Hammerschmidt K, Sennhenn-Reulen H, Freund AM, Fischer J. 2016 Motivational
- 27 shifts in aging monkeys and the origins of social selectivity. *Curr. Biol.* **26**, 1744–1749.
- 28
- 29 112. Brenman-Suttner DB, Yost RT, Frame AK, Robinson JW, Moehring AJ, Simon AF. 2020 Social
- 30 behavior and aging: A fly model. *Genes, Brain Behav.* **19**, e12598.
- 31
- 32 113. Rosati AG, Hagberg L, Enigk DK, Otali E, Emery Thompson M, Muller MN, Wrangham RW,
- 33 Machanda ZP. 2020 Social selectivity in aging wild chimpanzees. *Science (80-.).* **370**, 473–476.
- 34
- 35 114. Charles ST, Carstensen LL. 2010 Social and emotional aging. *Annu. Rev. Psychol.* **61**, 383–409.
- 36
- 37 115. Siracusa ER, Higham JP, Snyder-Mackler N, Brent LJN. 2022 Social ageing: exploring the drivers of
- 38 late-life changes in social behaviour in mammals. *Biol. Lett.* **18**, 20210643.
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Data Accessibility

The datasets supporting this article are provided as supplementary material.

Authors' Contributions

AD conceived the study. AD designed the study. AR, EP, PM, CC and MD performed the experiments. AR, EP, PM and CC carried out data acquisition. AD analysed the data. AD wrote the manuscript. AD secured funding. All authors gave final approval for publication.

Competing Interests

We have no competing interests.

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3 **Figure 1:** Experimental design. (a) Effect of various substrate (nutritive substrate: 5% oat gel, aversive
4 substrates: 0.6% NaCl + oat gel, 0.8% NaNO₃ + oat gel) on migration speed experiment as a function
5 of slime mould age (6 groups of different age were tested, from 1 to 94 weeks old). (b) Effect of
6 temperature (standard temperature : 25°C, extreme temperature : 10°C and 38.5°C) on migration speed
7 as a function of slime mould age (5 groups of different age were tested from 8 to 101 weeks old). (c)
8 Decision making under nutritive (2.5% oat gel vs 5% oat gel) and aversive conditions (0.4% NaCl +
9 5% oat gel vs 0.6% NaCl + 5% oat gel, 0.4% NaNO₃ + 5% oat gel vs 0.6% NaNO₃ + 5% oat gel) as a
10 function of slime mould age (5 groups of different age were tested, from 6 to 99 weeks old). (d)
11 Habituation to an aversive substance (0.6% NaCl) as a function of slime mould age (2 groups of
12 different age were tested : young slime moulds of 12 weeks and old ones of 74 weeks). (e) Effect of
13 dormancy on migration speed on a neutral (agar gel) and nutritive substrate (oat gel) as a function of
14 slime mould age (2 groups of different age were tested : old slime mould of 60 weeks and very old
15 ones of 100 weeks). (f) Fusion ability as a function of slime mould age (2 groups of different age were
16 tested: : relatively young slime moulds of 18 weeks and old ones of 75 weeks old). (g) Effect of fusion
17 on migration speed on a nutritive substrate (oat gel) as a function of slime mould age (2 groups of
18 different age were tested: young slime mould of 5 weeks and old ones of 54 weeks). (h) Detection of
19 chemical cues left by conspecifics (young and old) as a function of slime mould age (2 groups of
20 different ages were tested: young slime moulds of 3 weeks and old ones of 52 weeks).

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37 **Figure 2:** Mean migration speed as a function of age and substrate. Six groups of 10 large plasmodia
38 of different ages (1, 17, 32, 54, 74 and 94 weeks old) were used to conduct this experiment. The
39 substrates were made of oat gel (5%w/v) containing one of two aversive substances (Oat + NaCl (0.6%
40 w/v), Oat + NaNO₃ (0.8% w/v)) or not (Oat). N > 48 for each age and each substrate. Total numbers
41 of assays= 2396. Error bars indicate CI95.

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50 **Figure 3:** Mean migration speed and mean proportion of motionless slime moulds as a function of age
51 and temperature. Five groups of 10 large plasmodia of different ages (8, 24, 61, 81 and 101 weeks old)
52 were used to conduct this experiment. Three temperatures were tested: a standard one (25°C) and two
53 extremes (10°C and 38.5°C). The substrates were made of oat gel (5% w/v). All the slime moulds at
54 25°C migrated on the bridge so the proportion of motionless slime mould was equal to zero for all age
55 groups. N > 80 for each age and each temperature. Total numbers of assays= 3533. Error bars indicate
56 CI95.
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3 **Figure 4:** Decision making as a function of age and substrates. Five groups of 10 large plasmodia of
4 different ages (6, 22, 59, 79 and 99 weeks old) were used to conduct this experiment. Under non
5 aversive conditions (oat), the slime moulds were offered a choice between a high-quality oat gel (5%
6 w/v) and a low-quality one (2.5% w/v). Under aversive conditions (Oat+ NaCl or Oat+NaNO₃), the
7 slime moulds were given a choice between a high-aversive option (0.6% w/v) and a low-aversive
8 option (0.4% w/v). Decision making performance was computed as the distance covered on the best
9 bridge divided by the total distance. A value equal to 0.5 means that the slime mould distribution is
10 symmetric between the two bridges, a value equal to 1 or 0 means that the slime moulds migrated only
11 one the best or the worse bridge respectively. N > 235 for each age and each condition. Total numbers
12 of assays= 4206. Error bars indicate CI95.
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22 **Figure 5:** Learning as a function of age. (a) Migration speed on day 1. Two groups of 10 large
23 plasmodia of two different ages 12 and 74 weeks old were used to conduct this experiment. Slime
24 moulds were required to migrate on an oat gel bridge with an aversive substance NaCl (Oat+ NaCl)
25 (0.6% w/v, habituated treatment) or a plain oat gel bridge (Oat) without the repellent (control
26 treatment). Low values of speed indicate an aversive response. (b) Aversion index on day 4 computed
27 after the habituation phase. On day 4, all slime moulds (habituated and control) had to migrate either
28 on a bridge with the aversive substance or without. An aversion index closes to 0 indicates habituation
29 while values clearly above 0 indicate an aversion to the repellent. N = 50 for each age (young and old)
30 and each treatment (habituated and control). Total numbers of assays= 200. Error bars indicate 95%
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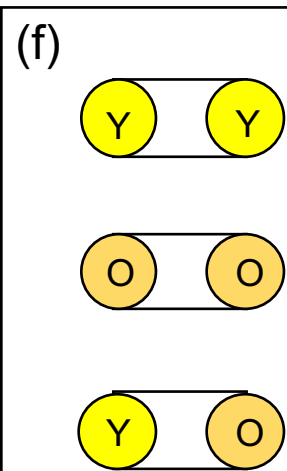
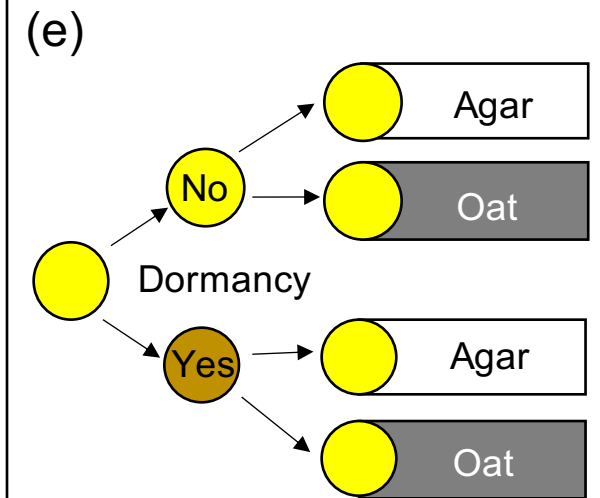
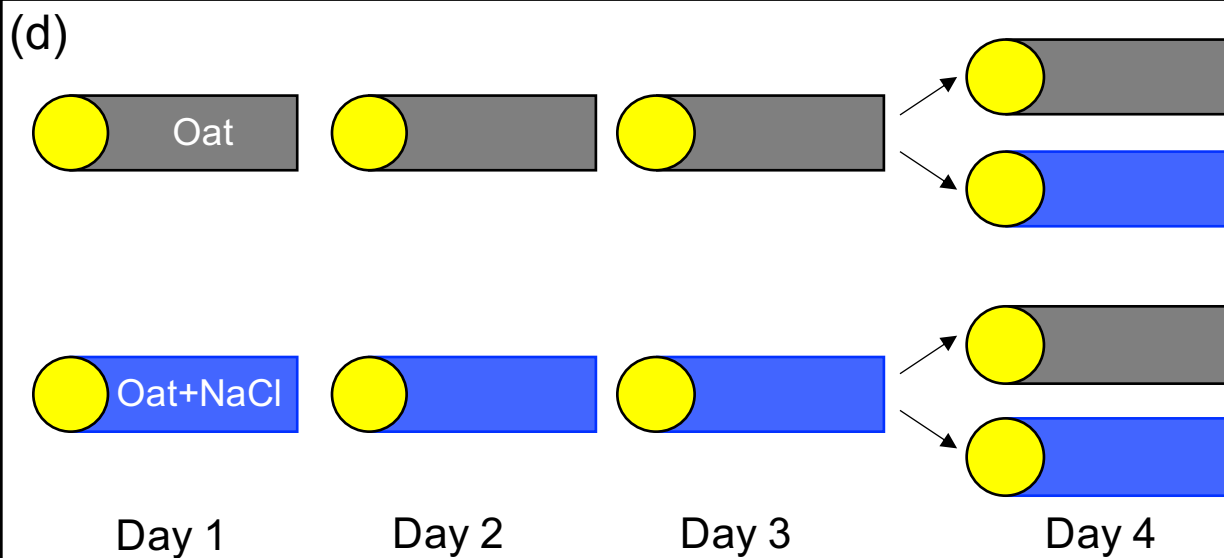
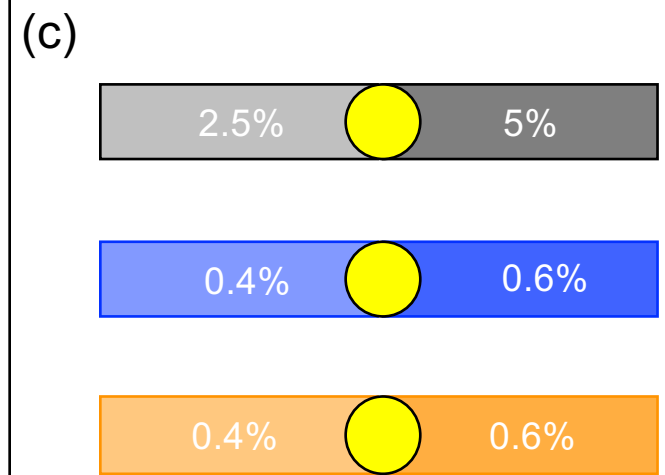
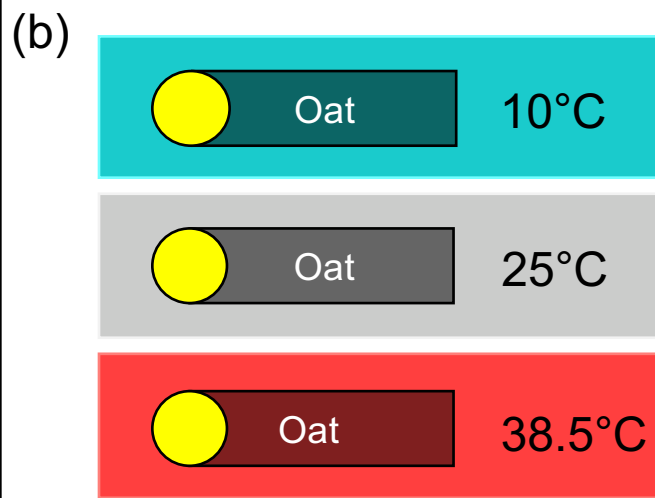
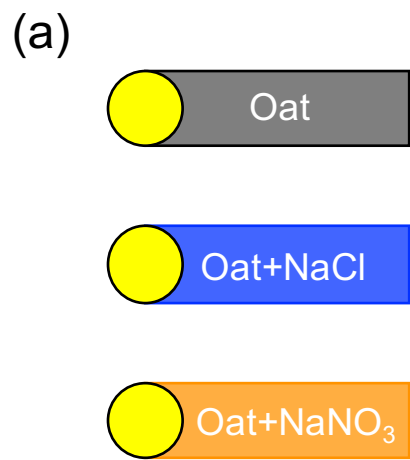
43 **Figure 6:** Mean migration speed following a dormancy period. Two groups of 10 large plasmodia of
44 two different ages 60 (old) and 100 (very old) weeks old were used to conduct this experiment. Before
45 the migration speed assays, slime moulds were either turn into sclerotia for a week and reinitiated
46 (dormancy: yes) or kept as plasmodia (dormancy: no). The substrates were made of oat gel 5%w/v
47 (Oat) or plain agar gel 1%w/v (Agar). N =80 for each age (old and very old) and each treatment
48 (dormancy yes or no). Total numbers of assays= 640. Error bars indicate CI95.
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56 **Figure 7:** Fusion as a function of age. Two groups of 10 large plasmodia of two different ages 18
57 (young) and 75 (old) weeks old were used to conduct this experiment. Young (old) slime moulds were
58 paired with an individual of the same age or older (younger). The bridge separating the two slime
59 moulds within a pair was made of plain agar gel 1% w/v. (a) Latency to membrane fusion as a function
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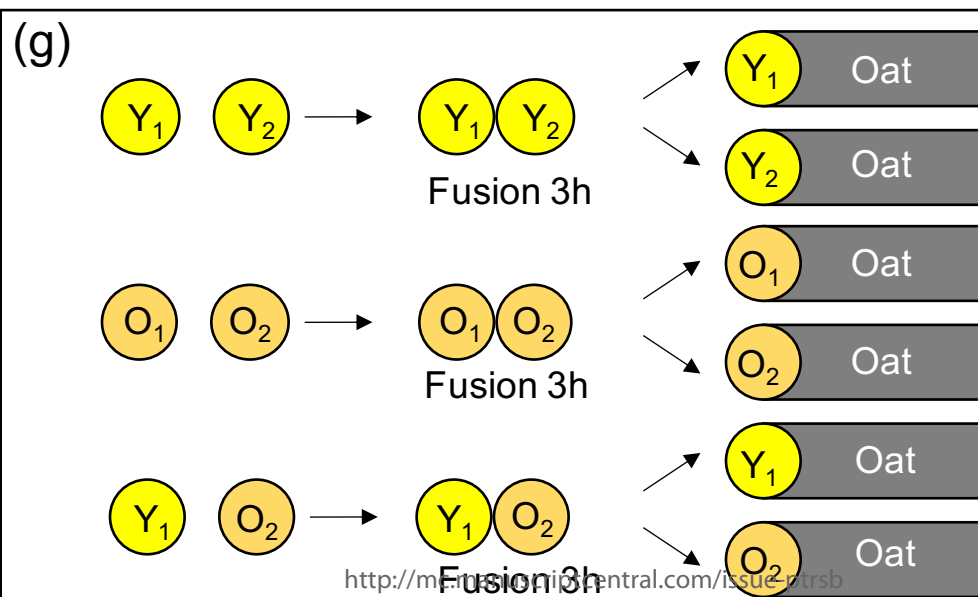
1 of age. The time of membrane fusion was recorded for each pair. (b) Latency to vein fusion as a
2 function of age. The time of vein connection following membrane fusion was recorded for each pair.
3 N=198, N=305 and N=202 for young/ young, young/old and old/old, respectively. Error bars indicate
4 CI95.
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11 **Figure 8:** Mean migration speed following fusion. Two groups of 10 large plasmodia of two different
12 ages 5 (young) and 54 (very old) weeks old were used to conduct this experiment. Before the migration
13 speed assays, slime moulds were allowed to fuse either with a slime mould of the same age or with a
14 slime mould of a different age. The substrates were made of oat gel 5%w/v (Oat). N =100 for each par
15 type (young/young, young/old, old/old). Total numbers of assays= 600. Error bars indicate CI95.
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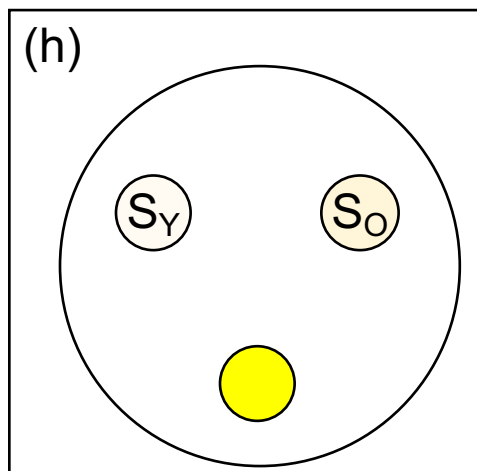
23 **Figure 9:** Detection of conspecifics as a function of age. Two groups of 10 large plasmodia of two
24 different ages 3 (young) and 52 (old) weeks old were used to conduct this experiment. Slime moulds
25 were offered a choice between two substrates previously explored by individuals of different ages. We
26 computed the proportion of slime moulds that reached each substrate first. A value equal to 0.5 means
27 that the slime moulds contacted the substrate explored by a young individual as often as the substrate
28 explored by a young individual (random choice). N = 300 for each age. The CI of the random choice
29 are delineated by shaded areas. Error bars indicate CI95.
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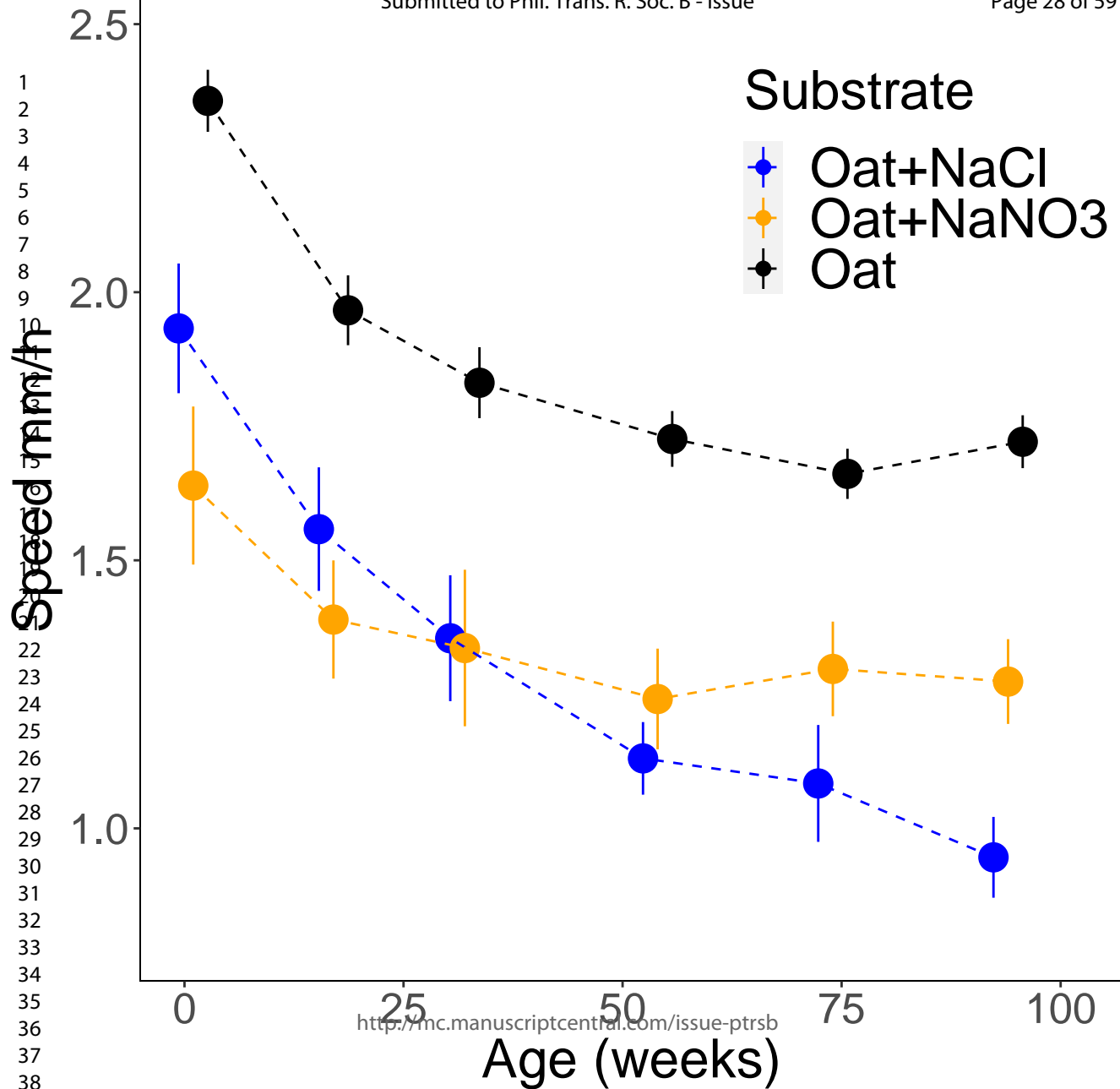
Y= Young
O= Old

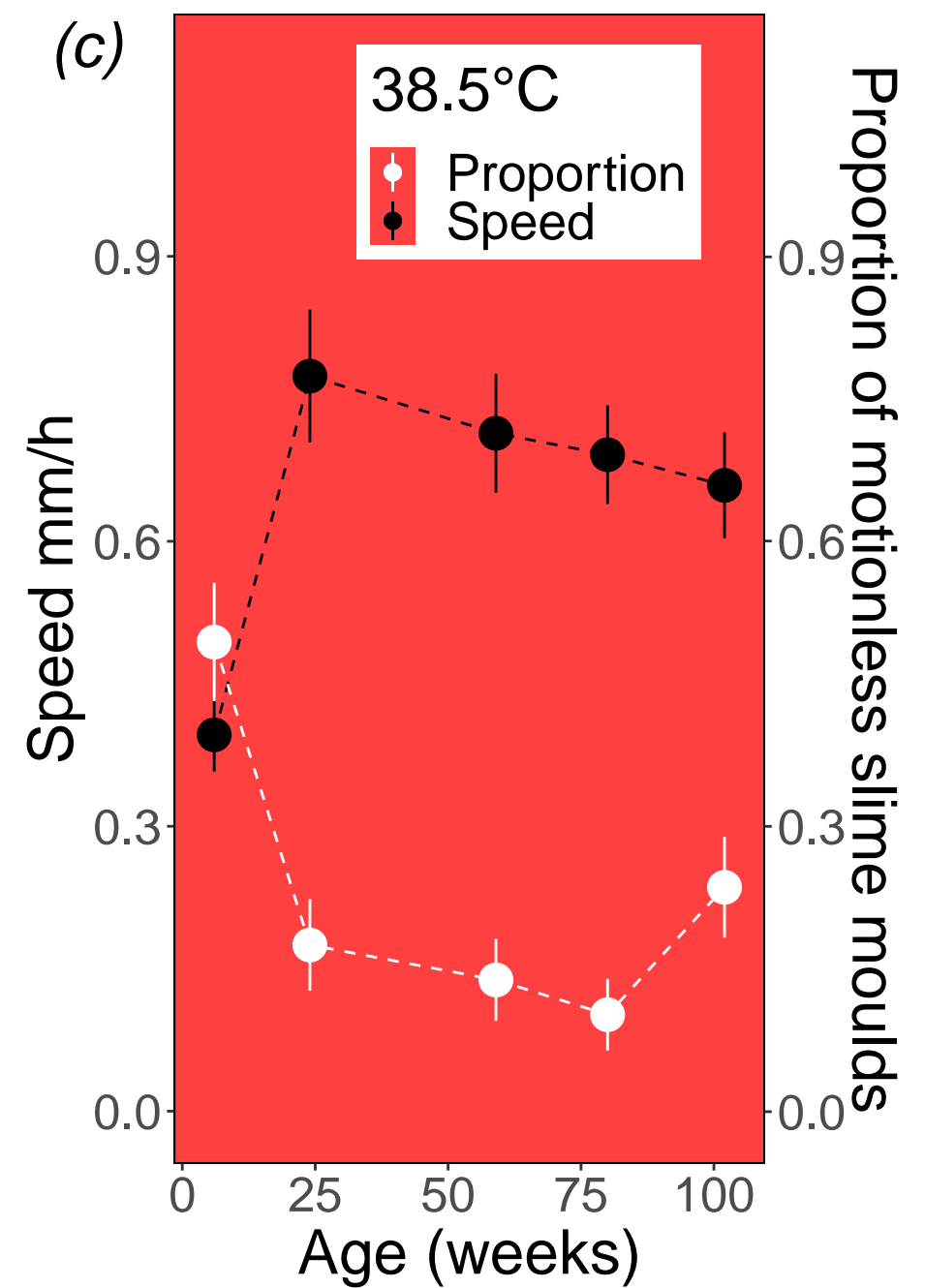
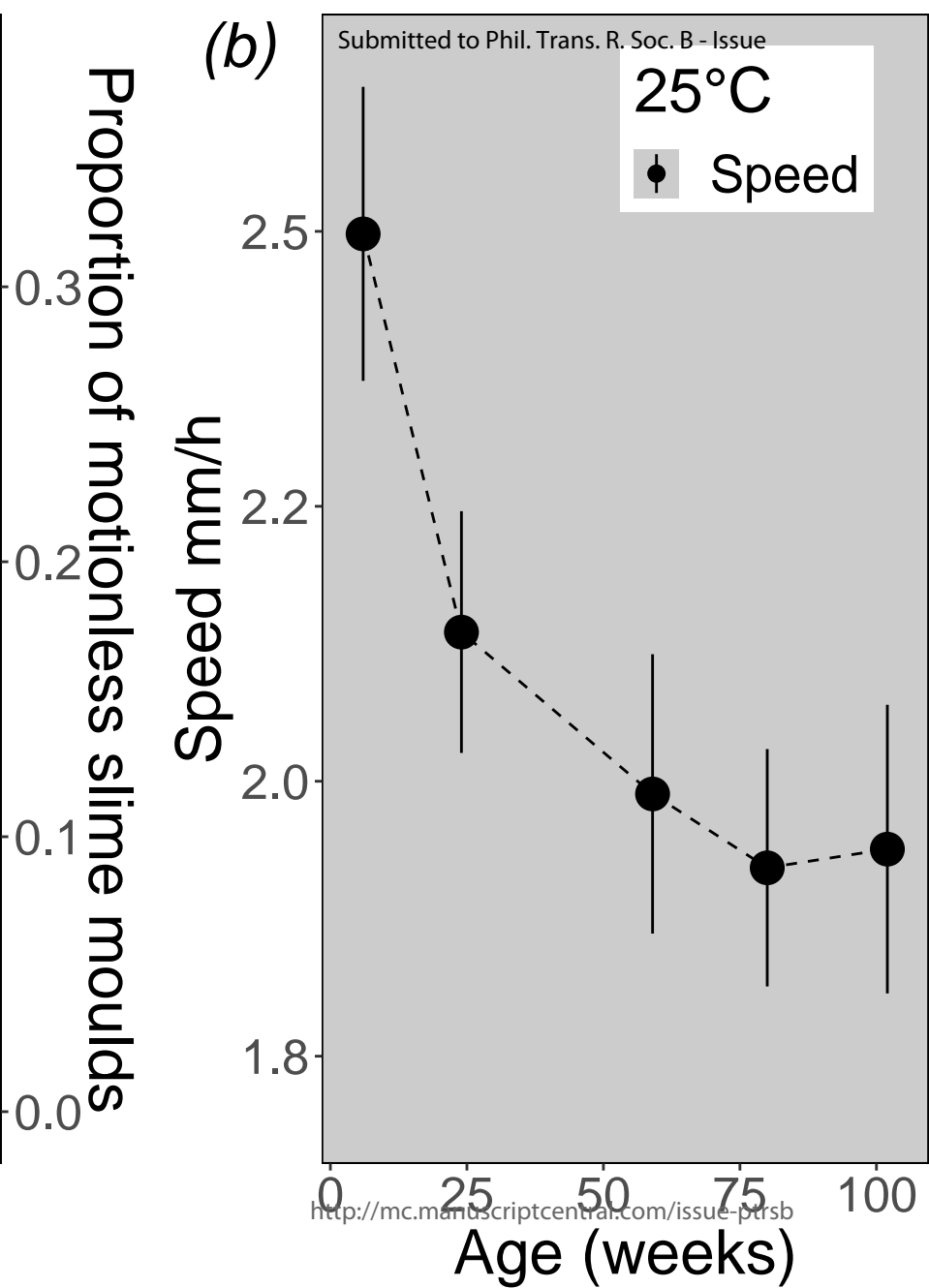
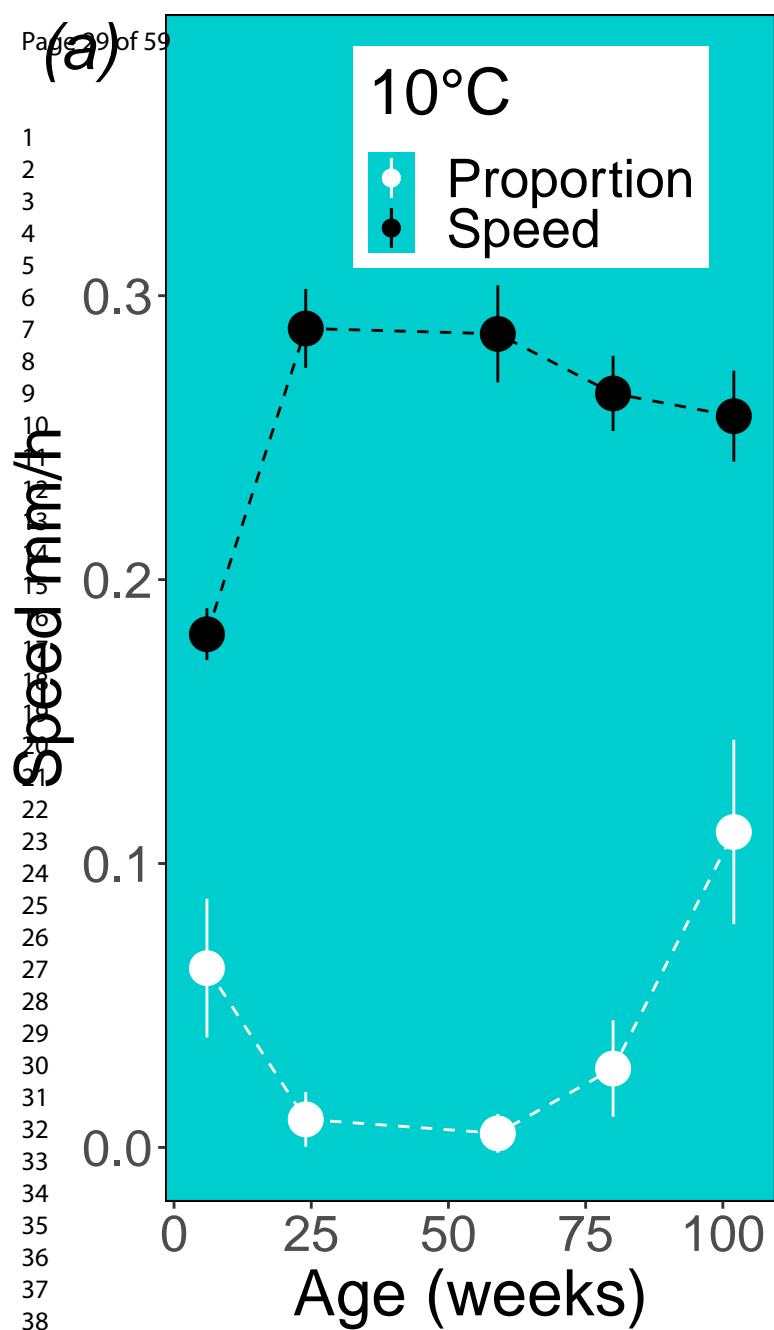


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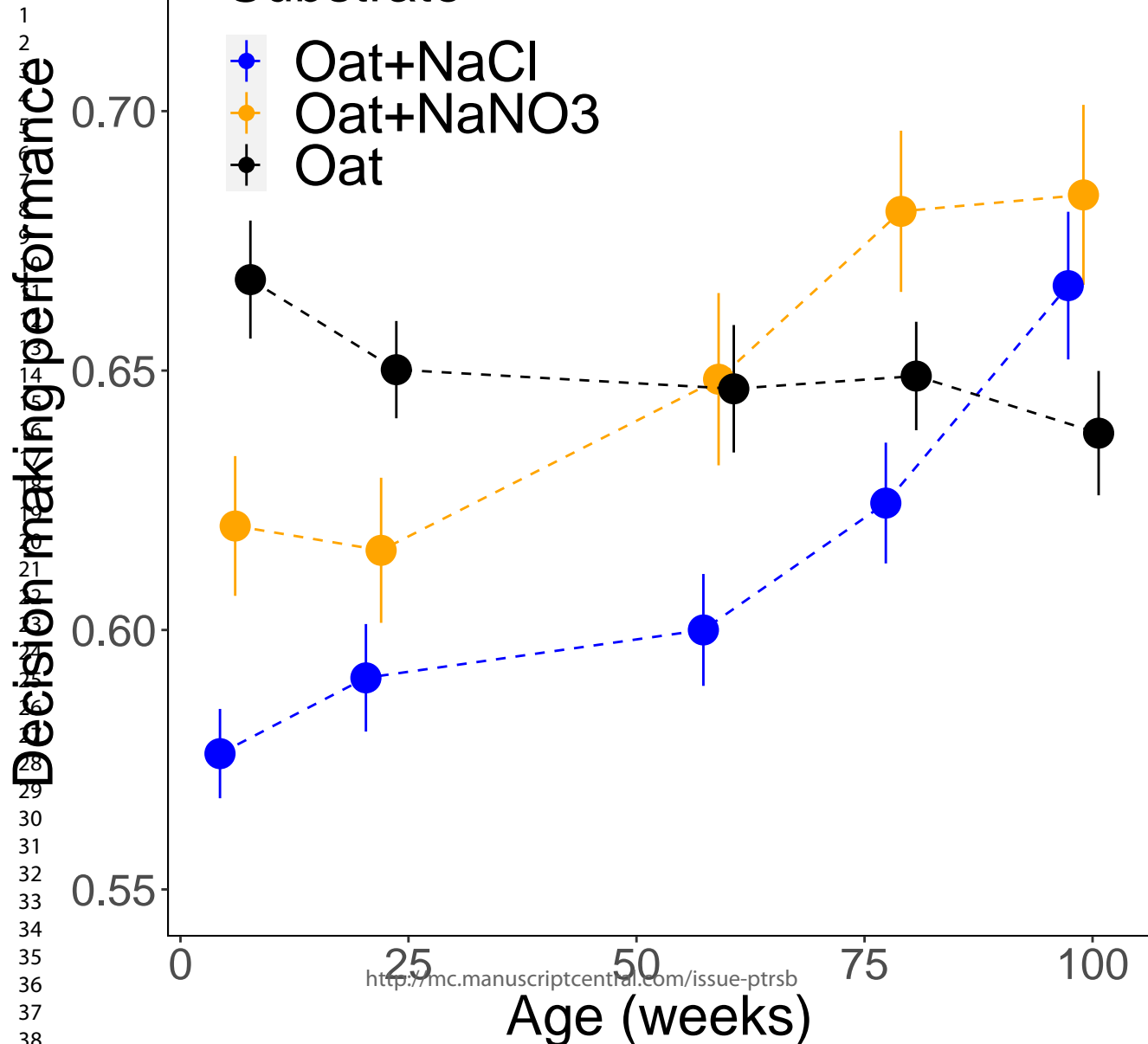
S_Y= Substrate from young
S_O= Substrate from old

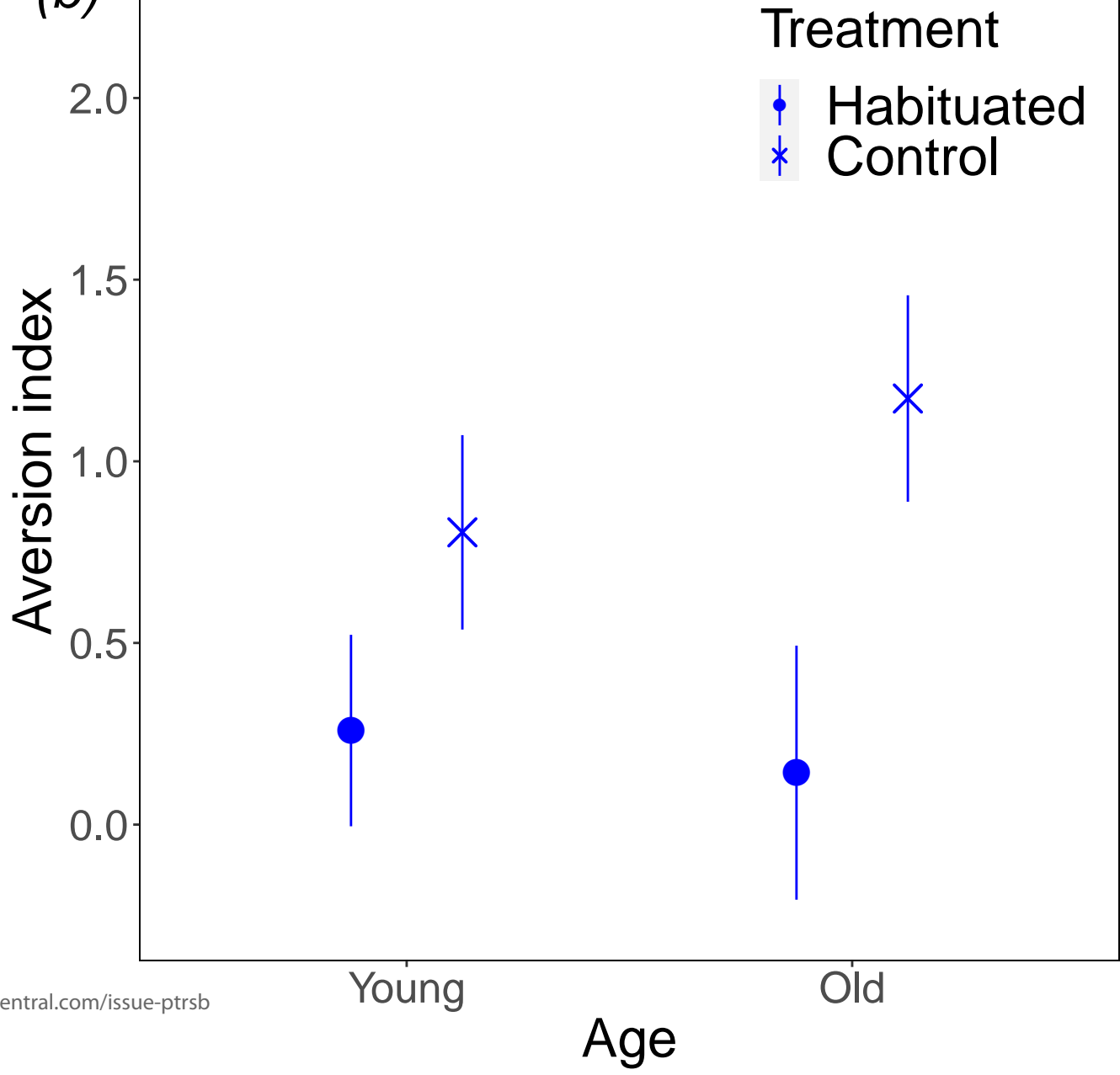
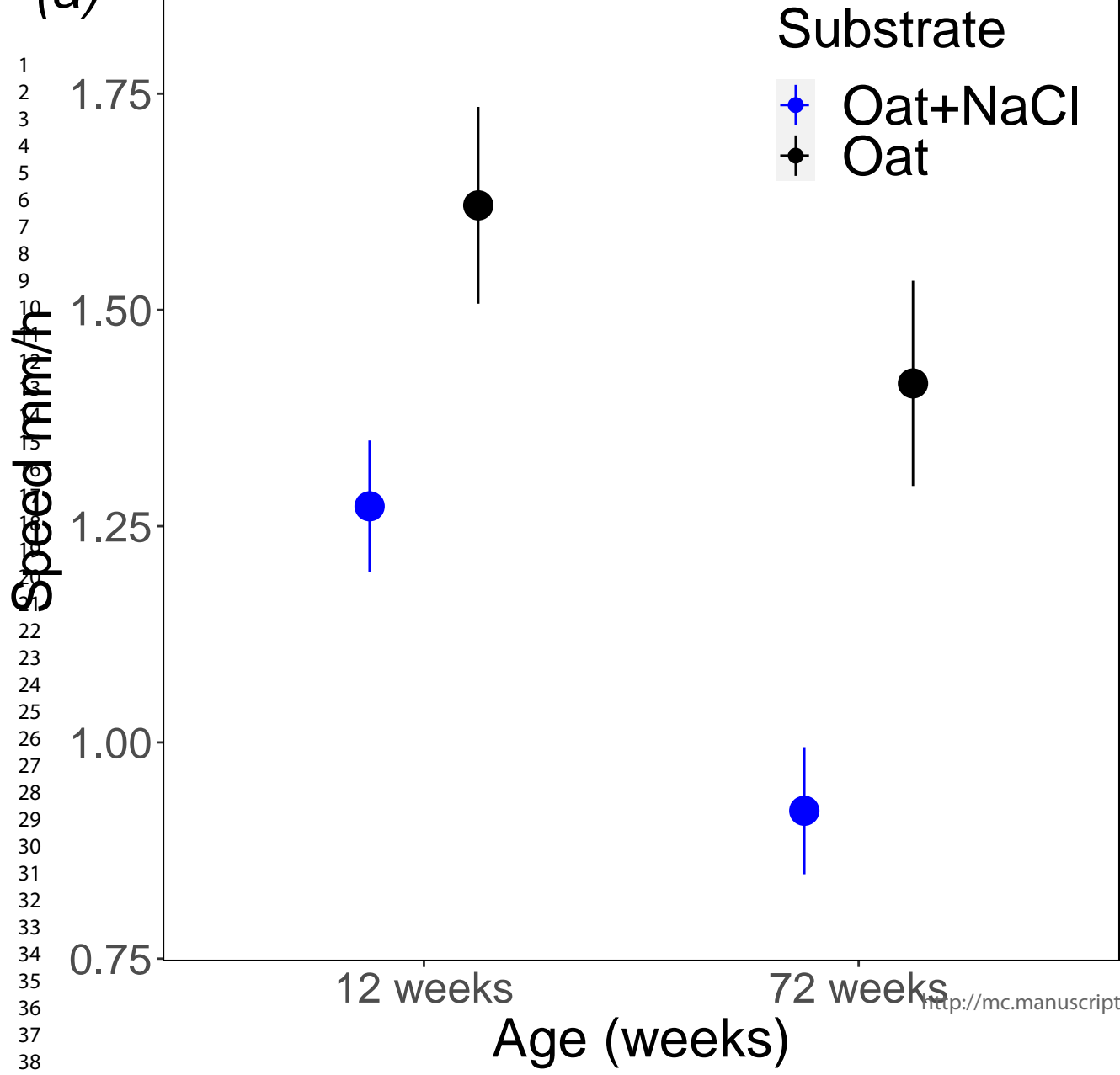




Substrate

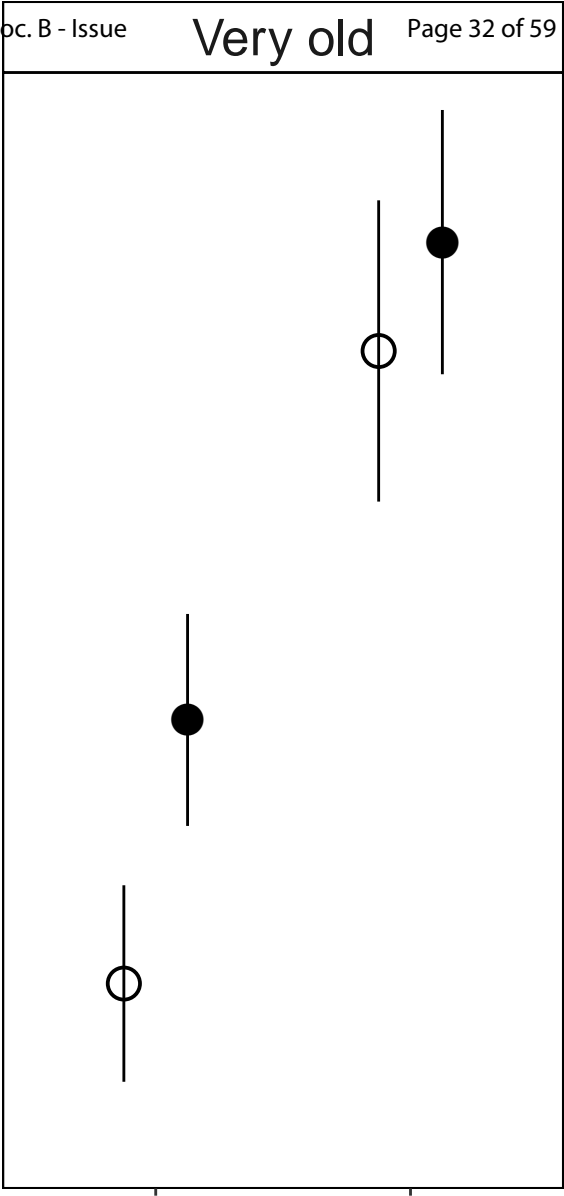
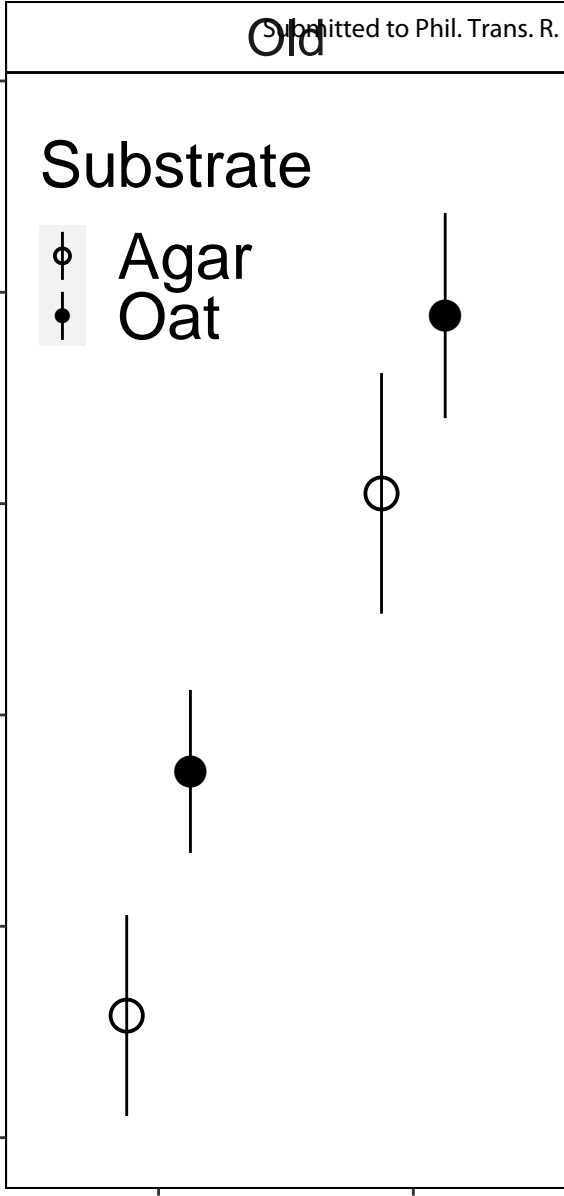
- Oat+NaCl
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- Oat



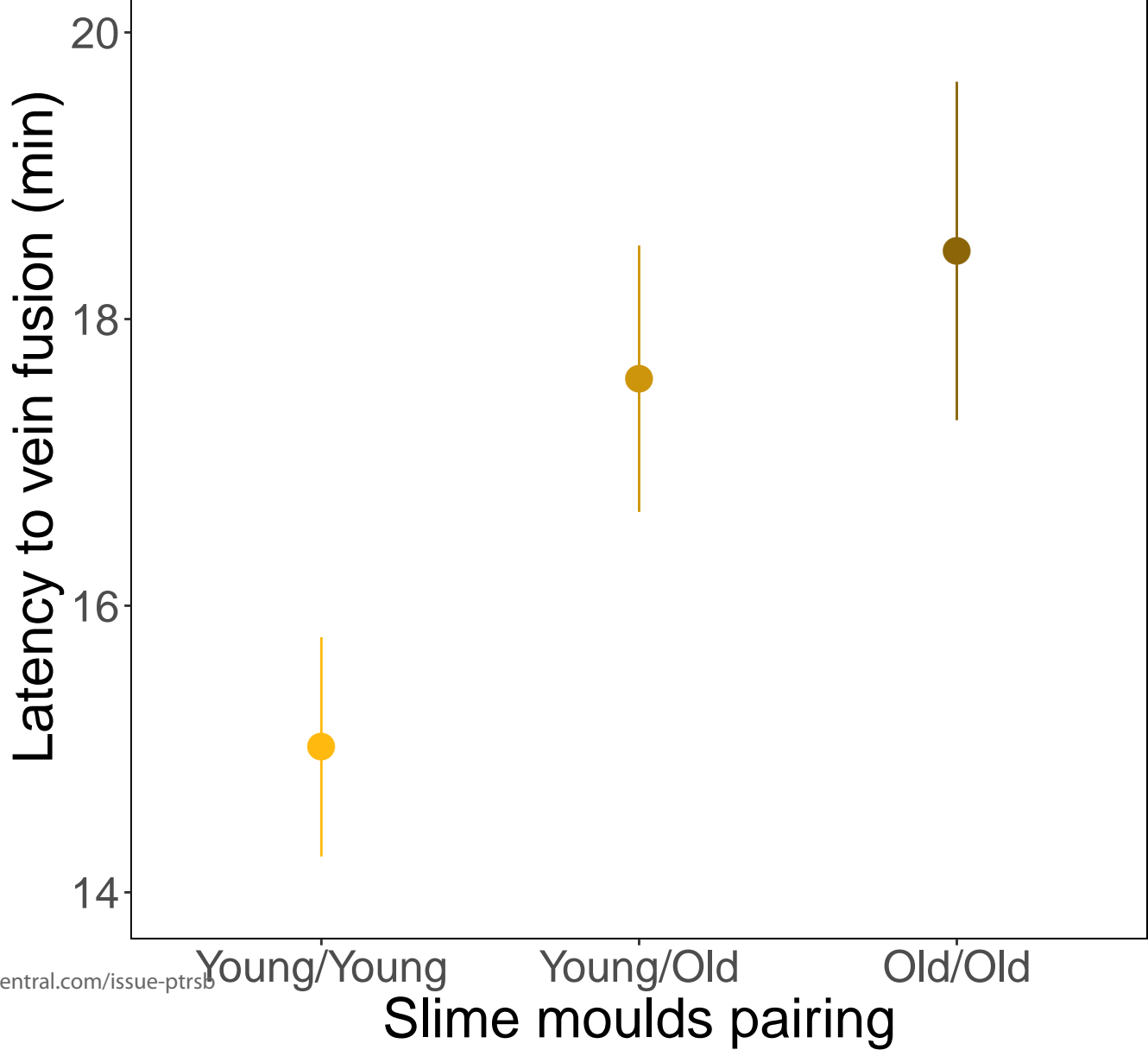
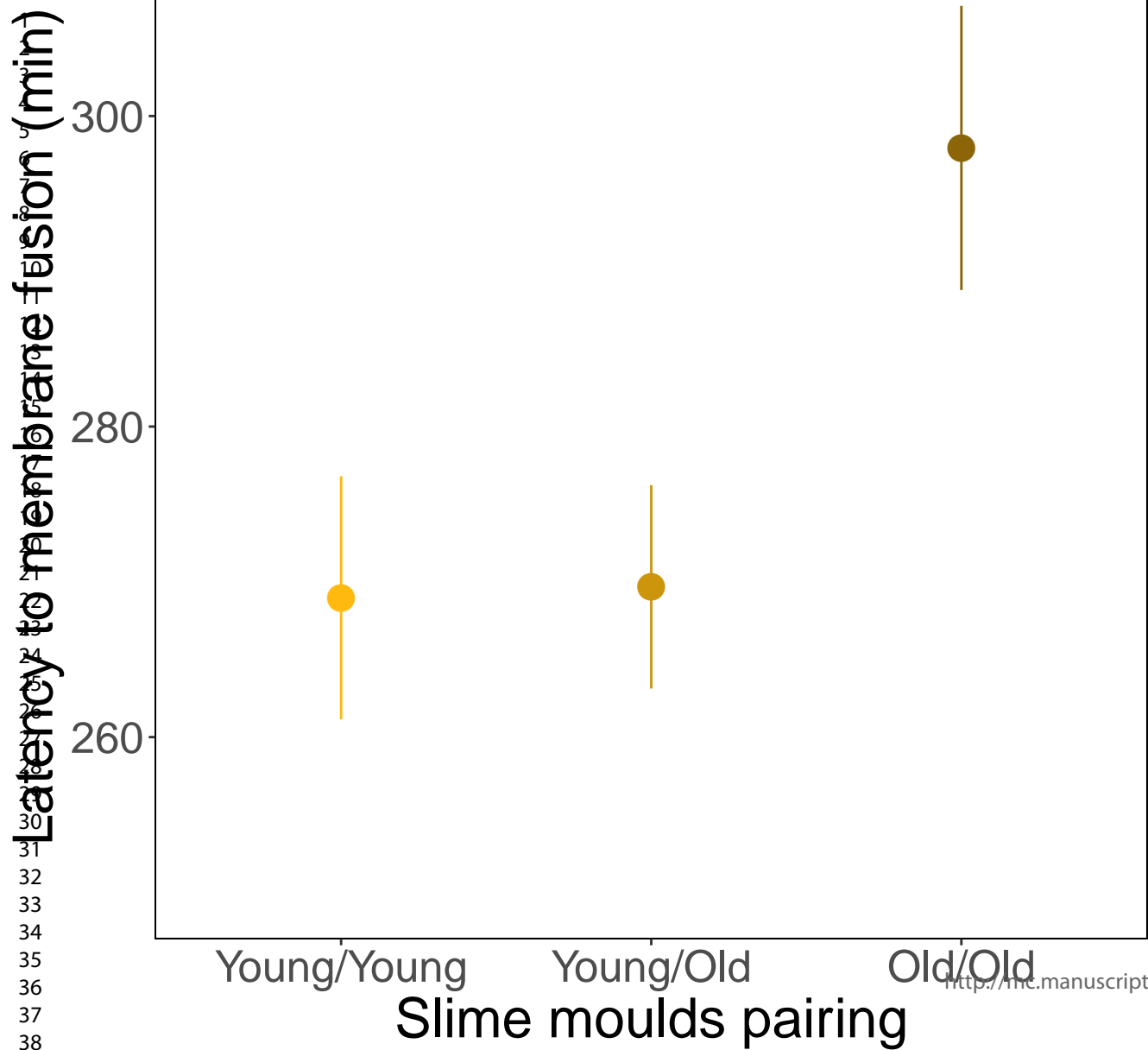


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Dormancy



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Fused with

-  A young slime mould
-  An old slime mould

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Speed mm/h

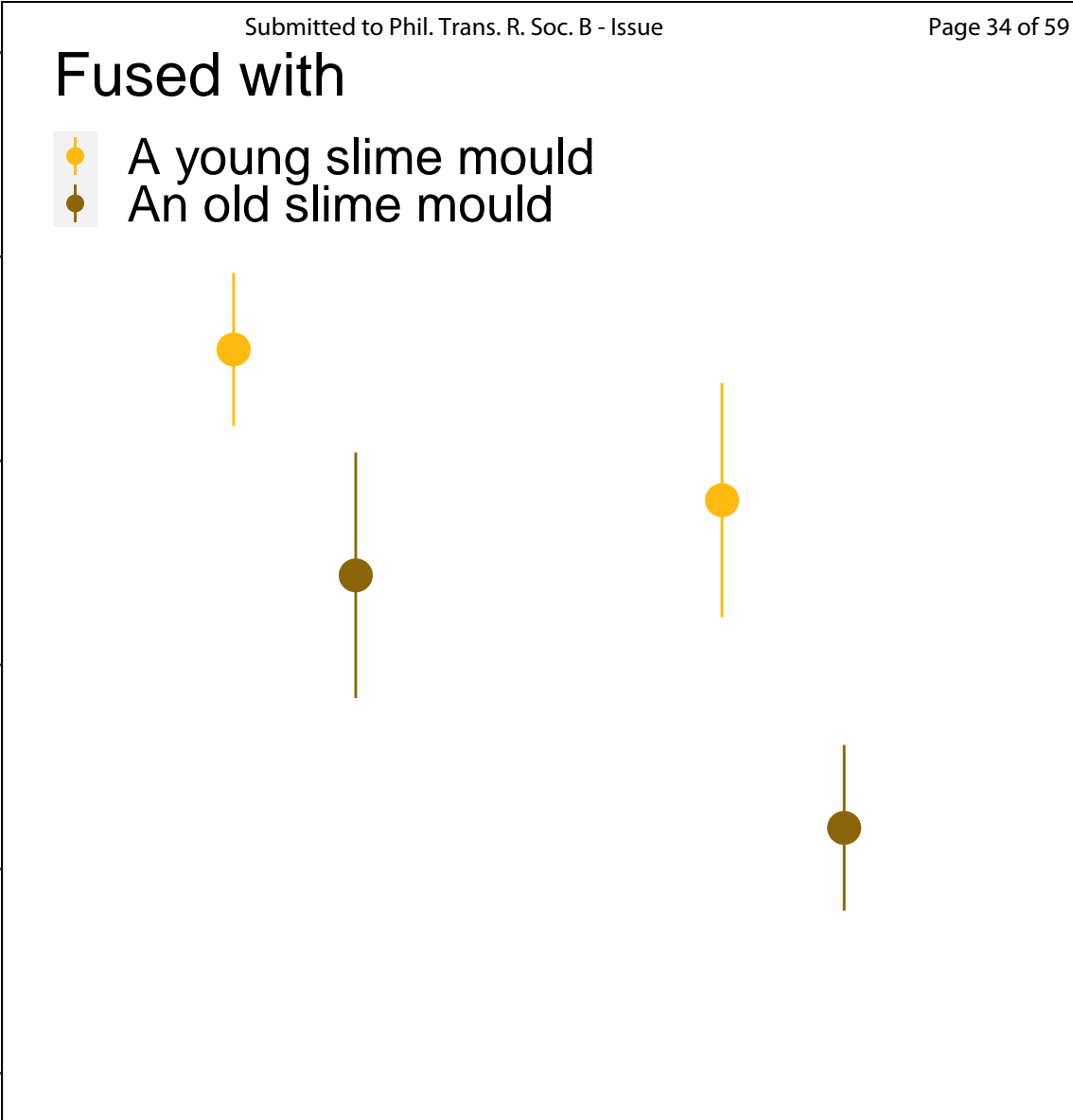
Young

Old

<http://mc.manuscriptcentral.com/issue-ptrsb>

Age

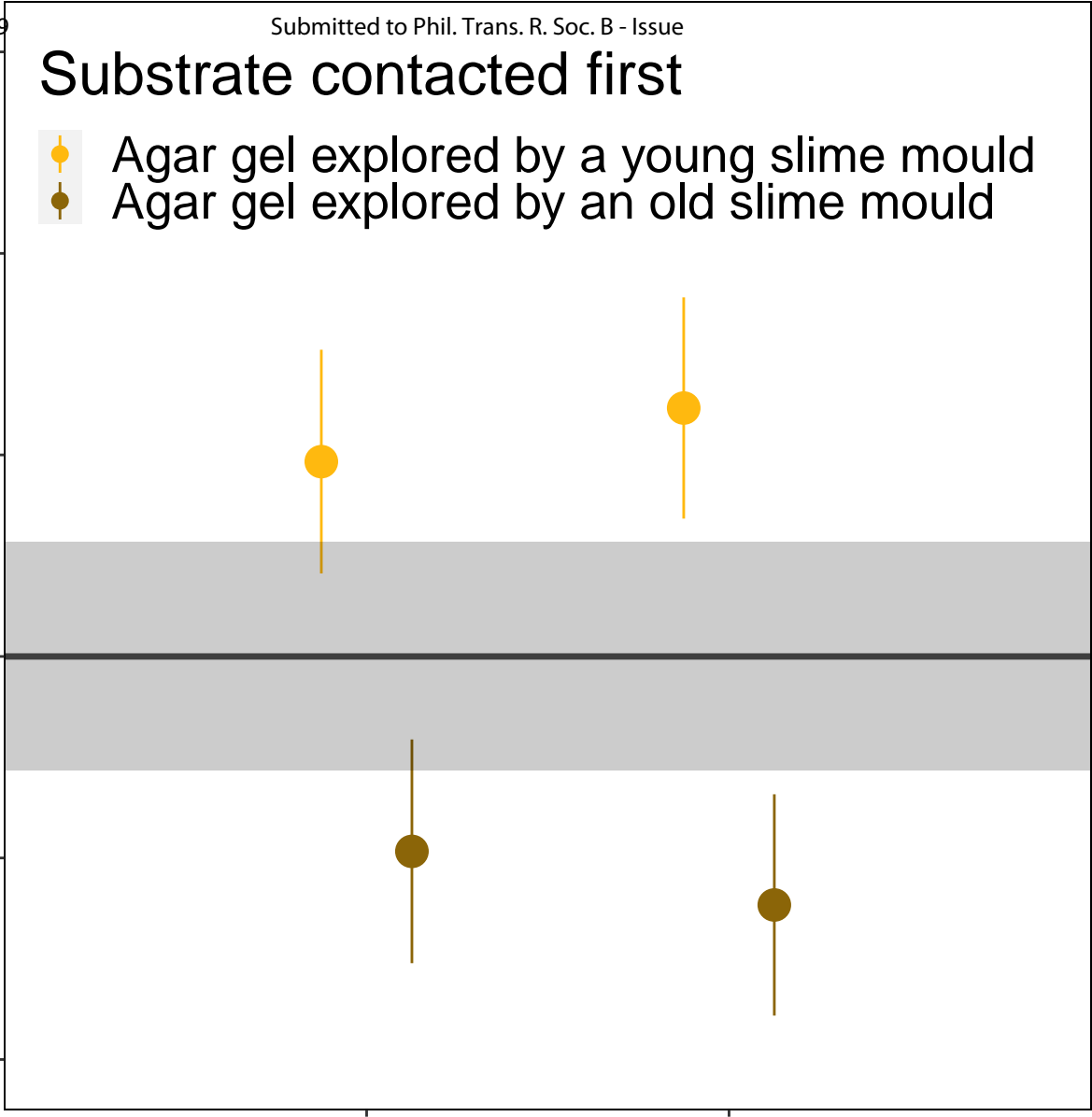
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Substrate contacted first

Agar gel explored by a young slime mould
Agar gel explored by an old slime mould

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Behavioural changes in slime moulds over time

**Rolland Angèle^{1†}, Pasquier Emilie^{1†}, Malvezin Paul^{1†}, Cassandra Craig^{1†},
Dumas Mathilde[†], Dussutour A[†]**

[†]Research Centre on Animal Cognition (CRCA), Centre for Integrative Biology (CBI), Toulouse University, CNRS, UPS, Toulouse 31062, France.

¹Contributed equally to the work

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4 Summary

5 Changes in behaviour over the lifetime of single cell organisms has primarily been investigated in
6 response to environmental stressors. However, growing evidence suggests that unicellular organisms
7 undergo behavioural changes throughout their lifetime independently of the external environment.
8 Here we studied how behavioural performances across different tasks vary with age in the acellular
9 slime mould *Physarum polycephalum*. We tested slime moulds aged from 1 week to 100 weeks. First,
10 we showed that migration speed decreases with age in favourable and adverse environments. Second,
11 we showed that decision making and learning abilities do not deteriorate with age. Third, we revealed
12 that old slime moulds can recover their behavioural performances if they go throughout a dormant
13 stage or if they fuse with a young congener. Lastly, we observed the response of slime mould facing a
14 choice between cues released by clone mates of different age. We found that both old and young slime
15 moulds are attracted toward cues left by young slime moulds. Although many studies have studied
16 behaviour in unicellular organisms, few have taken the step of looking for changes in behaviour
17 over the lifetime of individuals. This study extends our knowledge of the behavioural plasticity of single-
18 celled organisms and establish slime moulds as a promising model to investigate the effect of aging on
19 behaviour at the cellular level.

*Author for correspondence (audrey.dussutour@cnrs.fr).

[†]Present address: Research Centre on Animal Cognition, Centre for Integrative Biology, Toulouse University, CNRS, UPS, Toulouse 31062, France.

22 Introduction

23 One aim of this special issue of *Philosophical Transactions* is to understand collective
24 behaviour over the lifetime of individuals. In unicellular organisms, changes in collective behaviour
25 through time has been studied almost exclusively in response to environmental stressors. For instance,
26 it has been shown that bacteria [1,2] and cellular slime moulds [3–5] transit to new collective states
27 such as coordinated cellular migration or aggregation when nutrients become scarce [6]. On the
28 contrary, how collective behaviour changes with correlates of age in unicellular organisms
29 independently of the external environment remains an open question. The main reason for this might
30 be that, single cell organisms were mistakenly believed to be short lived and immune to ageing under
31 optimum growth conditions [7–11]. It has now been demonstrated that, some unicellular organisms
32 such as bacteria *Escherichia coli* and *Caulobacter crescentus*, ciliates *Paramecium* and *Tetrahymena*,
33 yeast *Saccharomyces cerevisiae*, cellular and acellular slime moulds *Dictyostelium discoideum* and
34 *Physarum polycephalum* undergo intrinsic changes over time that affect cell behaviour and physiology
35 [11–13]. In this paper, our aim is to understand how cell behaviour varies over the lifetime of
36 individuals in the acellular slime mould *P. polycephalum*. In contrast to the cellular slime mould *D.*
37 *discoideum* in which the vegetative state consists of solitary amoebas that divide about every 4 h when
38 food is plentiful, in *P. polycephalum*, the vegetative state is a unique large cell containing a large
39 collection of nuclei that divide about every 8 h and share a common cytoplasm. Acellular slime moulds
40 are particularly attractive organisms to study collective behaviour changes over time in unicellular
41 organisms for several reasons.

42 First of all, although they are not collective *per se*, acellular slime moulds embrace most of the
43 key principles of collective behaviour (reviewed in [14]): synchronization and coordination [15–18],
44 positive and negative feedbacks [19], variability [20–22], symmetry breaking [20,23], phase transition
45 [24], redundancy [14], information transfer [25,26] capacity to make complex decisions [27–30]. In
46 addition, due to its internal dynamic, *P. polycephalum* is often regarded as a collection of nonlinear
47 oscillators which are interconnected, often coupled and partially dependent on each other (reviewed in
48 [16]). Furthermore, similarly to more traditional model systems in collective behaviour such as ant
49 colonies or fish schools, its behaviour has successfully been simulated using a multi-agent approach
50 [31,32]. In these models, *P. polycephalum* behaviour relies on particle like agents that follow simple
51 rules but are capable collectively to generate self-organized amoeboid movement and to construct
52 optimized networks [31,32].

53 Second, despite being unicellular, acellular slime moulds can produce seemingly complex
54 behaviours. Indeed, since the seminal contribution of Toshiyuki Nakagaki twenty years ago [33], *P.*
55 *polycephalum* has become an essential model organism for studying problem-solving in non-neural
56 systems [32–37]. Past experiments have shown that acellular slime moulds can find the shortest path
57 in a maze [33,38], build optimized networks to connect several food sources [34], anticipate events

1 58 [39], learn to ignore irrelevant stimuli [40,41], encode memory in their environment [42] or in their
2 morphology [43], interact with their congeners [21,44], optimize nutrient intake [28,45], make optimal
3 decisions [29,46,47], etc. *P. polycephalum*'s behaviour relies on its self-organized internal architecture
4 which consists of a transport network of interconnected tubes [48]. The tubes contract and relax
5 periodically, causing the cytoplasm to flow back and forth, a phenomenon termed 'shuttle streaming'
6 [49]. *P. polycephalum* can migrate at a speed of up to few millimetres per hour through the interplay
7 of intracellular flow, adhesion and rhythmic contractions of the tubes [49–51]. The frequency and the
8 amplitude of the contractions depend on external stimuli and as a result, *P. polycephalum* is capable of
9 altering its shape and motion as a function of a variety of external stimuli such as chemicals [52–54],
10 light [55], temperature [56–58], humidity [44], gravity [59] or substrate distortion [46].
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19 Third, acellular slime moulds are easy to track through time and relatively long-lived. In
20 unicellular organisms, lifetime can either be chronological if we consider the time a cell remains viable
21 before dividing, or replicative if we consider the number of times a cell divide before it dies [9].
22 Tracking division is usually difficult as it requires to follow and distinguish individual cells. *P.*
23 *polycephalum* offers the possibility to consider both chronological and replicative time simultaneously.
24 During its development, the zygote undergoes synchronous mitotic divisions every 8 to 12 hours but
25 without cytokinesis to form a single multinucleated cell, called a plasmodium [60,61]. Hence, the cell
26 grows over time but there is no cell division. A plasmodium reared on solid medium by routine serial
27 subculture under laboratory condition can be maintained from a few weeks to several months [62,63].
28 The life span of the plasmodium is in part genetically determined as different strains have different
29 lifespans and subclones derived from a single plasmodium age in a coordinated manner, i.e death
30 occurs at approximately the same time [62,64,65].
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39 Fourth, *P. polycephalum* provides unique possibilities for experimental manipulations [66,67].
40 The plasmodium can extend up to hundreds of square centimetres. It can be severed into viable and
41 structurally similar yet smaller plasmodia. Upon contact, genetically identical plasmodia can fuse with
42 each other to form a single plasmodium. In response to environmental stressors, the plasmodium can
43 enter a resting stage called sclerotium and remain dormant for years without deterioration. During the
44 sclerotization process, the plasmodium lose 50% of their total protein content together with 40% of
45 their DNA and 65% of their RNA [60]. After transfer to a humid and nutritive medium, a sclerotium
46 can revert back to a mobile plasmodium within 24 h. Plasmodial cultures can be easily initiated from
47 sclerotia after up to 3 years. Interestingly, numerous authors have reported that, a sclerotium issued
48 from a seemingly ageing plasmodium may produce a vigorous plasmodium again, a form of
49 "rejuvenescence". Following a period of growth this secondary plasmodium will undergo senescence
50 again. This cycle of growth and senescence can be repeated several times and, thus, cultures can be
51 maintained over many generations.
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1 93 Lastly, *P. polycephalum* displays senescence, which is defined as a loss of fitness during ageing.
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3 94 A plasmodium can be maintained for months during which no change is apparent until a reproducible
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5 95 decline in viability occurs [62]. Senescence in the plasmodial stage is characterized by morphological
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7 96 change [62], reduction of growth rate [62,68], reduced cytoplasmic flow [64], loss of yellow pigment
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9 97 [64], accumulation of polyploid nuclei [69], high level of mitochondrial DNA fragmentation [68],
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11 98 decreased replication of mitochondrial DNA [63], increase in nuclear size and DNA content [69,70].
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13 99 Senescence ends with the fragmentation of the plasmodium into several small spherical structures with
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15 100 a concomitant lysis of the plasmodium [64]. Longevity in slime moulds is not affected by most
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17 101 environmental factors with the exception of high temperature [71]. Senescence in slime moulds has
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19 102 been mainly studied at the cellular level. The potential effects of ageing on the plasmodium behaviour
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21 103 remained to be investigated.

22 104 In this paper, we studied how slime mould behaviour changes over time. First, we explored the
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24 105 effect of age on migration speed in nutritive and adverse environments. Second, we investigated if
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26 106 decision making and learning performances changed with age. Third, we tested if older slime moulds
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28 107 were able to recover after a dormant period or after fusing with younger clones. Lastly, we examined
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30 108 if slime moulds were able to discriminate cues from congeners of different age.

31 109 **Methods**

32 33 110 **(a) Species studied and rearing conditions**

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35 111 *Physarum polycephalum*, also called the acellular slime mould, belongs to the supergroup Amoebozoa
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37 112 and the class Myxomycetes. In nature, slime moulds are found on organic substrates like tree bark or
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39 113 forest soil where they feed on microorganisms such as bacteria or fungi. *P. polycephalum* vegetative
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41 114 morph is a vast multinucleated cell named plasmodium. We used a strain of *P. polycephalum* : LU352
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43 115 kindly provided by Professor Dr Wolfgang Marwan (Max Planck Institute for Dynamics of Complex
44
45 116 Technical Systems, Magdeburg, Germany). 80 large plasmodia were initiated from sclerotia at
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47 117 different time to obtain slime moulds of different age. In this paper, by age of the slime mould, we
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49 118 understand the time which elapses from the moment of the inoculation of a sclerotium onto a nutritive
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51 119 medium. The oldest plasmodia were kept after this study and are still being cultivated in our laboratory.
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53 120 They are currently more than 2 years old and none of them has died so far. In previous reports,
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55 121 plasmodium lifespan has been shown to vary from one month to one year, depending on the strain [62-
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57 122 64]. Hence, we considered as “old”, slime molds which were over one year old and “very old” slime
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59 123 molds which were almost two years old.

60
61 124 Plasmodia were reared on a 1% w/v (weight/volume) agar medium with rolled oat flakes (Quaker Oats
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63 125 Company®) in Petri dishes (90mm Ø). They were kept in the dark in a thermoregulated chamber at a
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65 126 temperature of 20 degrees Celsius and a humidity of 80% from Monday to Friday. They were

1 127 transferred every day on a new agar medium on which was spread a single layer of rolled oat flakes.
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3 128 During the weekends, slime moulds were unfed and kept at 12°C. One day before each experiment, all
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5 129 plasmodia were transferred on a food medium consisting of finely ground oat flakes (5% w/v) mixed
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7 130 with 1% agar gel, hereafter referred as “oat gel”.

9 131 **(b) Migration speed on various substrates as a function of slime moulds age**

11 132 The aim of this experiment was to investigate how age affects the slime mould migration speed on
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13 133 various substrates (Figure 1a). Six groups of 10 large plasmodia of different ages (1, 17, 32, 54, 74
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15 134 and 94 weeks old) were used to conduct this experiment. Circular samples (10 mm Ø) were cut from
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17 135 each plasmodium and gently placed in contact with an oat gel bridge (35 mm long, 10 mm wide) in an
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19 136 experimental arena (Petri dish 12mm*12mm). The bridge either contained an aversive substance: NaCl
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21 137 (100mM, 0.6% w/v) (Oat + NaCl bridge) or NaNO₃ (100mM, 0.8% w/v) (Oat + NaNO₃ bridge) or not
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23 138 (Oat). Experimental arenas housed 8 bridges and were kept in a thermoregulated chamber at a
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25 139 temperature of 25 degrees Celsius. After about 8 hours of incubation, the distance travelled by the
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27 140 slime moulds on each bridge was measured with a ruler. We computed the migration speed as the
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29 141 distance travelled divided by the time spent in contact with the bridge. A minimum of 48 samples were
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31 142 tested for each substrate and each age group, leading to total of 2396 assays.

31 143 **(c) Migration speed at different temperatures as a function of slime moulds** 32 33 144 **age**

35 145 Slime moulds have been shown to suffer both from low and high temperature which affect the shuttle
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37 146 streaming and therefore locomotion [72]. The aim of this experiment was to investigate how age affects
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39 147 the slime mould migration speed under different temperatures (Figure 1b). Five groups of 10 large
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41 148 plasmodia of different ages (8, 24, 61, 81 and 101 weeks old) were used to conduct this experiment.
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43 149 Circular samples (10 mm Ø) were cut from each plasmodium and gently placed in contact with an oat
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45 150 gel bridge (35 mm long, 10 mm wide) in an experimental arena (Petri dish 12mm*12mm).
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47 151 Experimental arenas housed 8 bridges and were kept in thermoregulated chambers at a temperature of
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49 152 10, 25 or 38.5°C degrees Celsius. 25°C is a standard temperature to rear *Physarum polycephalum* while
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51 153 10°C and 38.5°C represents extremes conditions. After about 8 hours of incubation, the distance
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53 154 travelled by the slime moulds on each bridge was measured with a ruler. We computed the migration
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55 155 speed as the distance travelled divided by the time spent in contact with the bridge. Numerous slime
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57 156 moulds did not move at all during the experiments at extreme temperatures (10 or 38.5°C), they were
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59 157 not taken into account to compute speed but we calculated the proportion of motionless slime moulds
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158 for each age group and each temperature. A minimum of 80 samples were tested for each temperature
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and each age group, leading to total of 3533 assays.

(d) Decision making ability as a function of slime moulds age

Slime moulds have been shown to succeed in selecting the best option when offered multiple alternatives [22,27,45,73]. To investigate how age affects slime mould ability to make a decision, we studied how a slime mould distributed itself between two agar gel bridges containing or not an aversive substance (NaCl or NaNO₃) (Figure 1c). Under non aversive condition, the slime moulds were offered a choice between a high-quality oat gel (5% w/v) and a low-quality one (2.5% w/v). Under aversive condition, the slime moulds were given a choice between a high-aversive option (0.6% w/v NaCl or NaNO₃) and a low-aversive option (0.4% w/v NaCl or NaNO₃). Five groups of 10 large plasmodia of different ages (6, 22, 59, 79 and 99 weeks old) were used to conduct this experiment. Circular samples (10 mm Ø) were cut from each plasmodium, placed between two bridges (35 mm long, 10 mm wide) in experimental arenas (Petri dish 12mm*12mm). Experimental arenas housed 4 binary choices and were kept in a thermoregulated chamber at a temperature of 25 degrees Celsius. After about 8 hours of incubation, the distance travelled (in mm) on each bridge was measured with a ruler. We computed a decision-making performance as the distance covered on the best bridge (high quality or low aversive substrate) divided by the total distance. A minimum of 235 samples were tested for each age and each binary choice, leading to total of 4206 assays

(e) Learning ability as a function of slime moulds age

Slime moulds have been shown to be capable of habituation, a simple form of learning, when repeatedly exposed to an innocuous aversive substance [40,74]. To investigate how age affects slime moulds' ability to learn, we studied how slime moulds habituate to an aversive substance [25,40]. A bridge crossing experiment adapted from [40] was conducted to habituate the slime mould to an aversive substance (NaCl 100mM, 0.6% w/v) (Figure 1d). We used two groups of 10 large plasmodia of two different ages 12 and 74 weeks old. Before starting the habituation experiment, to accustom the slime moulds to the experimental set-up, 10 circular samples (Ø 10 mm) were cut from each plasmodium and introduced in an experimental arena (Petri dish 12mm*12mm). The samples were then connected to an oat gel bridge (35 mm long, 10 mm wide). After a day, the slime moulds had covered the bridge, and a circular sample was cut from the bridge and transferred to new experimental arenas to start the habituation. On day 1 of the habituation, half of the samples were offered an oat gel bridge containing NaCl (substrate: Oat+NaCl, treatment: habituated, N=50 for each age group) while the remaining half had to cross a bridge without NaCl (substrate: Oat, treatment: control, N=50 for each age group). The following two days (day 2 and day 3), a circular sample was cut from each bridge daily and transferred to another arena where they were offered a new bridge. On day 4, once the habituation training was completed, we cut one or two circular samples from each bridge and transferred them to a new arena. Half of the habituated and control samples were offered a bridge containing NaCl (substrate: Oat+NaCl) while the remaining half had to cross a bridge without NaCl

(substrate: Oat). Thus, on day 4 the control group is either facing a bridge containing NaCl for the first time (N=30 for each age group) or a bridge without NaCl (N=30 for each age group) while the habituated group is either facing a bridge containing NaCl for the fourth time (N=30 for each age group) or a bridge without NaCl (N=30 for each age group). Every day, after about 8 hours of incubation, the distance travelled (in mm) on each bridge was measured with a ruler. Data collected on day 1 allowed us to confirm that slime moulds showed a clear aversive behaviour toward the NaCl while data collected on day 4 enable us to test if slime mould learn to ignore the aversive substance. On day 4 we synthesized the results with an aversion index HI and CI as in [41].

$$HI = \left| \frac{1}{N} \sum_{i=1}^N \frac{HON_i - \overline{HO}}{sd(\overline{HO})} \right| \quad \text{or} \quad CI = \left| \frac{1}{N} \sum_{i=1}^N \frac{CON_i - \overline{CO}}{sd(\overline{CO})} \right|$$

HON, CON, HO and CO are the habituated (H) and control slime moulds (C) on Oat+NaCl (ON) and Oat (O) respectively. Using those indexes allowed us to normalize each variable value corresponding to the treatments HON and CON by the mean and the standard deviation of their respective control HO and CO. Values clearly above 0 indicate an aversion towards the repellent, whereas values close to zero indicate habituation to the repellent *i.e.* slime moulds react the same way to Oat+NaCl and Oat.

(f) Recovery ability following dormancy as a function of slime moulds age

To investigate how age affects slime mould ability to recover, we studied how fast old slime moulds migrate after a dormancy period (Figure 1e). We used two groups of 10 large plasmodia of two different ages 60 and 100 weeks old, referred as “old” and “very old”. To initiate the transition from plasmodia to sclerotia, we cut 2 circular samples from each plasmodium ($\varnothing = 20$ mm), placed each of them on a moist paper filter ($\varnothing = 145$ mm) in a petri dish ($\varnothing = 145$ mm) and kept them in a thermoregulated chamber at a temperature of 30 degrees Celsius for two days to dry. The sclerotia were then stored for a week before being re-initiated to obtain new plasmodia. A week later, we then had 40 large plasmodia, 20 were one week old and originated from old and very old plasmodia (treatment: dormancy) and 20 were old and very old (treatment: no dormancy). Circular samples ($\varnothing 10$ mm) were cut from each plasmodium and gently placed in contact with an oat gel bridge (nutritive substrate) or an agar gel bridge (agar 1%w/v, non-nutritive substrate) in an experimental arena (Petri dish 12mm*12mm). Experimental arenas housed 8 bridges and were kept in a thermoregulated chamber at a temperature of 25 degrees Celsius. After about 8 hours of incubation, the distance travelled by the slime moulds on each bridge was measured with a ruler. We computed the migration speed as the distance travelled divided by the time spent in contact with the bridge. A minimum of 80 samples were tested for each age group (old, very old) and each treatment (dormancy or not), leading to total of 640 assays.

(g) Fusion as a function of slime moulds age

Fusion constitutes a defining feature of the lifestyle of slime moulds. To investigate how age affects slime mould ability to fuse, we studied how fast two slime moulds fuse as function of their respective age (Figure 1f). We used two groups of 10 large plasmodia of two different ages 18 and 75 weeks old, referred as “young” and “old”. Circular samples (10 mm Ø) were cut from each plasmodium and gently placed in contact with an agar gel bridge (15 mm long, 15 mm wide) in an experimental arena (Petri dish 12mm*12mm). We placed a sample at each extremity of the bridge and tested the following pairs: young/young (N=220), young/old (N=330) and old/old (N=220). Experimental arenas housed 8 pairs of slime moulds. They were kept in a thermoregulated dark room at a temperature of 21 degrees Celsius. We conducted behavioural scan observations using a flash light to record the latency to membrane and vein fusion. Membranes were considered as fused when we could not see the delimitation between the two slime moulds while veins were considered as fused when we could discern a vein going from one slime mould to the other. The latency between two scans was less than 5 minutes. Pairs that did not fuse after 9h or that fused with their neighbouring slime moulds in the arenas were removed from the analysis (young/young: 26 out of 220, young/old: 25 out of 330 and old/old: 18 out of 220)

(h) Recovery ability following fusion as a function of slime moulds age

Slime mould fusion could provide the potential for cell recovery through resource sharing. Recently, it was shown that a **learned** behaviour can be transferred during fusion [25]. In this experiment, we measure migration speed after fusion as a function of slime moulds age (Figure 1g). We used two groups of 10 large plasmodia of two different ages 5 and 54 weeks old, referred as “young” and “old”. Circular samples (10 mm Ø) were cut from each plasmodium and gently placed by pairs in an experimental arena (Petri dish 12mm*12mm). Each experimental arenas housed 4 pairs. We tested the following pairs: young/young (N=100), young/old (N=100) and old/old (N=100). Fusion was allowed by bringing the slime moulds into contact for 3 hours (see Vogel and Dussutour 2016). After the fusion, the slime moulds were gently separated at the point of contact using a spatula. Then, all slime moulds were required to cross an oat gel bridge (35 mm long, 10 mm wide). Experimental arenas housed 8 bridges and were kept in a thermoregulated chamber at a temperature of 25 degrees Celsius. After about 8 hours **of** incubation, the distance travelled by the slime moulds on each bridge was measured with a ruler.

(i) Detection of conspecifics as a function of slime moulds age

Slime moulds can sense and respond to chemical cues released in the environment by conspecifics in a foraging context [21,44]. They release calcium while foraging which is attractive to other slime moulds [21]. In this experiment we monitored the directional movement response evoked in slime

1 263 moulds in the presence of substrates previously explored by clone mates of different age (Figure 1*h*).
2
3 264 We used two groups of 10 large plasmodia of two different ages 3 and 52 weeks old, referred as
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5 265 “young” and “old”. Circular Petri dishes (diameter, $\text{\O} = 90$ mm) containing a layer of 1% w/v agar gel
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7 266 were used as experimental arenas. Once the agar in the Petri dish had set, three holes were punched (\O
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9 267 = 10 mm, inter- distance = 20 mm). One hole was filled with a young and an old slime mould ($\text{\O} = 10$
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11 268 mm) sitting on 5% w/v oat gel. The other two holes were filled with experimental substrates ($\text{\O} = 10$
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13 269 mm) which consisted in agar gel explored for 24 h by well-fed young and old slime mould. To obtain
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15 270 the experimental substrates, a slime mould was allowed to cover completely an agar gel while being
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17 271 fed with oat flakes (making sure that the food was never in contact with the agar substrate, see [21] for
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19 272 details). Then we removed the slime mould, and rinsed the agar substrate with distilled water just before
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21 273 the experiment. The slime mould would typically explore its environment by expanding in all
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23 274 directions for a short distance to finally migrate in a specific direction, eventually contacting one
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25 275 experimental substrate. The experimental substrate that was reached first was taken to imply a positive
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27 276 response (*i.e.*, a relative preference for the cues enclosed in the experimental substrate over the
28
29 277 alternative). For each assay, we recorded which substrate was contacted first and the latency to reach
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31 278 each substrate. We replicated each binary choice 300 times with old and young slime moulds.
32
33 279 Experiments were recorded using digital cameras (EOS 70D, Canon) which took a picture every 5 min

31 (j) Statistical analyses

32 280
33 281 All statistics were performed with RStudio (Version 1.2.1335). To assess the difference in the various
34
35 282 parameters measured between the treatments, we used linear mixed models (LMM) or generalised
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37 283 mixed models (GLMM) (function lmer or glmer). The package lme4 [75] was used for all mixed
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39 284 models. The lmerTest and Car packages were then used to run Type III analysis of variance on the
40
41 285 calculated LMMs and GLMMs, extracting p-values for F-tests and Wald-tests. Assumptions for all
42
43 286 LMMs were checked using standard procedure: diagnostic of quantile-quantile normal plots and
44
45 287 Shapiro-Wilk test. The models were fitted by specifying the fixed effects (explanatory variables)
46
47 288 depending on the experiment and a random effect: the plasmodium identity. The dependent variables
48
49 289 that did not fit linear model requirements were transformed using the “bestNormalize” function
50
51 290 (“bestNormalize” package). Continuous variables were centred and scaled if needed. The outcomes of
52
53 291 all the models are presented in the supplementary information (Table S1-S13)

54 Results

57 (a) Migration speed on various substrates as a function of slime moulds age

58 293
59 294 In the first experiment, we investigated if slime moulds migration speed is affected by age on different
60
295 substrates. Slime moulds migrated faster on plain oat gel than on oat gels containing an aversive
296 substance (LMM, substrate $F=67.01$, $p<0.001$, table S1, figure 2). For all substrates, migration speed

297 decreased as age increased (LMM, age $F=80.52$, $p<0.001$, table S1, figure 2). On plain oat gel,
298 migration speed declined drastically until the slime moulds were 30 weeks old then decreased gently.
299 On oat gel containing NaNO_3 , migration speed reached a plateau when the slime moulds were over 30
300 weeks old. On oat gel containing NaCl , migration speed declined continuously with age (LMM age *
301 substrate $F=28.08$, $p<0.001$, table S1, figure 2). Hence, NaNO_3 was the most aversive substance for
302 young slime moulds while for old slime moulds it was NaCl .

303 **(b) Migration speed at different temperatures as a function of slime moulds** 304 **age**

305 In the second experiment, we examined if slime moulds' migration speed was affected by age at
306 different temperatures. Slime moulds migrated faster at standard temperature than at cold or hot
307 temperature (GLMM, temperature $\chi^2=4724.85$ $p<0.001$, table S2, figure 3). As previously, at standard
308 temperature, migration speed declined drastically with age at the beginning then decreased gently when
309 the slime moulds were over 25 weeks old. At both low and high temperatures, the speed was the lowest
310 for young slime moulds (8 weeks old), then increased until the slime moulds reached an intermediate
311 age between 24 and 61 weeks old and finally decreased slightly with age (GLMM, age $\chi^2=5.67$
312 $p=0.017$, age * temperature $\chi^2=43.26$ $p<0.001$, table S2, figure 3). The proportion of motionless slime
313 moulds was also affected by age (GLMM, age $\chi^2=10.11$ $p<0.001$, Table S3, figure 3) and temperature
314 (GLMM, temperature $\chi^2=200.47$ $p<0.001$, table S3, figure 3). The youngest (8 weeks old) and oldest
315 slime moulds (101 weeks old) remained most often motionless at both extreme temperatures with the
316 youngest being more vulnerable to cold and the oldest to heat (GLMM, age * temperature $\chi^2=39.58$
317 $p<0.001$, table S3, figure 3).

318 **(c) Decision making ability as a function of slime moulds age**

319 In the third experiment, we estimated slime moulds' ability to make a decision as a function of age and
320 substrate. As previously, slime moulds migrated faster on plain oat gel than on oat gels containing an
321 aversive substance (GLMM, substrate $\chi^2=451.80$ $p<0.001$, table S4) and for all substrates migration
322 speed decreased as age increases (GLMM, age $\chi^2=5.91$ $p<0.001$, age * substrate $\chi^2=18.88$ $p<0.001$,
323 table S4). On average, slime moulds had better performances under non aversive conditions (GLMM,
324 substrate $\chi^2=141.34$ $p<0.001$, table S5, figure 4). However, under aversive conditions, decision making
325 performance increased with age while it remained constant under non aversive conditions (GLMM,
326 age $\chi^2=4.22$ $p=0.040$, age * substrate $\chi^2=111.82$ $p<0.001$, table S5, figure 4). Hence, young slime mould
327 had better performances under non aversive condition while it was the contrary for old slime moulds.

328 **(d) Learning ability as a function of slime moulds age**

329 In the fourth experiment, we investigated if slime moulds' ability to habituate to an aversive substance
330 depended on age. On day 1, slime moulds migrated faster on plain oat gel than on oat gels containing

1 331 NaCl (LMM, substrate $F=75.86$ $p<0.001$, table S6, figure 5a) and migration speed was higher for
2 332 young than for old slime moulds (LMM, age $F=15.27$ $p=0.001$, age * substrate $F=3.76$ $p=0.054$, table
3 333 S6, figure 5a). On day 4, habituated and control slime moulds were tested for habituation and were
4 334 required to cross a bridge containing NaCl. Control slime moulds showed a strong aversive behaviour
5 335 and a high aversion index. In contrast, habituated slime moulds, encountering NaCl for the fourth time,
6 336 showed no aversive behaviour and an aversion index close to zero (LMM, treatment $F=23.54$ $p<0.001$,
7 337 table S7, figure 5b). Hence, they learned to ignore the aversive substance. Slime moulds age did not
8 338 affect their learning performance (LMM, age $F=2.15$ $p=0.145$, age*treatment $F=1.92$ $p=0.169$, table
9 339 S7, figure 5b)

19 341 **(e) Recovery ability as a function of slime moulds age**

21 342 In the fifth experiment, we tested if slime moulds recovered their original migration speed after a
22 343 dormancy period. Slime moulds migrated faster on oat gel than on agar gels (LMM, substrate $F=30.10$
23 344 $p<0.001$, table S8, figure 6) regardless of age and treatment (LMM, substrate * age $F=0.11$ $p=0.741$,
24 345 substrate * treatment $F=2.54$ $p=0.111$, table S8, figure 6). Slime moulds that were turned into a dormant
25 346 state and reinitiated were significantly faster than their counterparts that remained as plasmodia (LMM,
26 347 treatment $F=59.52$ $p<0.001$, table S8, figure 6). **Age of the dormant slime mould had no effect on speed**
27 348 **following dormancy** (treatment * age, $F=0.09$ $p=0.772$, table S8, figure 6).

34 349 **(f) Fusion as a function of slime moulds age**

35 350 In the sixth experiment we examined if the fusion process was affected by age. The latency for the
36 351 membrane to fuse was shorter for pairs of young slime moulds or mixed pair (young/old) than for pairs
37 352 of old ones (LMM, age $F=3.63$ $p=0.034$ table S9, figure 7a). Vein fusion, following membrane fusion,
38 353 also occurred earlier in pair of young slime moulds than in the other pairs (LMM, age $F=4.21$ $p=0.030$,
39 354 table S10, figure 7b).

45 355 **(g) Recovery ability following fusion as a function of slime moulds age**

46 356 In the fifth experiment, we tested if old slime moulds recovered their original migration speed after
47 357 fusing with young ones. **Young slime moulds were faster** than old slime mould when they were allowed
48 358 to fuse with a slime mould of the same age (LMM, age $F=19.68.52$ $p<0.001$, table S11, figure 8).
49 359 However, old slime moulds, that were allowed to fuse with a young one, were significantly faster than
50 360 their counterparts that fused with an old one (LMM, age*pair type $F=33.84$ $p<0.001$, table S11, figure
51 361 8). On the contrary young ones that were allowed to fuse with an old slime mould were slower than
52 362 their counterparts that fused with a young one.

(h) Detection of conspecifics as a function of slime moulds age

In the last experiment, we investigated if slime moulds could perceive a difference in cues left by slime moulds of different ages. As previously shown, young slime moulds were faster than old ones (GLMM, age $\chi^2=14.44$ $p<0.001$, table S12, figure 9). Both young and old slime moulds contacted first the substrate explored by a young slime mould significantly more often than the substrate explored by an old one (GLMM, observed vs expected: $\chi^2=5.67$ $p=0.017$, table S13, figure 9).

Discussion

In many organisms, ageing is accompanied by deficits in behavioural performance. In this study, we were able to maintain a culture of slime moulds for more than two years and track their behaviour throughout time. We revealed that old slime moulds move slower than young ones on neutral, nutritive and aversive substrates in all our experiments. We confirmed that aversive substrates slowed down slime moulds regardless of age [25,41,76,77]. Sodium is known to decrease migration rate in slime moulds via a depolarization of the membrane potential [78]. An interesting observation was that sodium chloride had a stronger effect than sodium nitrate on old slime moulds while it was the opposite for young slime moulds. In many eukaryotic cells, high extracellular NaCl increases reactive oxygen species (ROS), causes DNA damage and promotes senescence [79,80]. Given that old slime moulds already suffered from molecular damages due to senescence (effects listed in the introduction [62–64,68–70]), NaCl might have amplified those damages. Interestingly, old slime moulds were better at avoiding high concentration of NaCl or NaNO₃ than young slime moulds, suggesting that they were indeed more susceptible to chemical stressors and actively avoided them. As a matter of fact, we did not observe any significant differences in decision abilities when slime moulds were facing two food sources of different quality.

We then revealed that migration speed depended on age under extreme temperature. Temperature is known to be one of the main factors affecting the shuttle streaming in slime moulds. The contraction period decreases from 13min at 1°C to 1.26 min at 30°C and then increases past 32°C [57,58,81,82]. At 42°C, the shuttle streaming is totally arrested and the membrane exhibits alterations [81]. In our experiment, all slime moulds were slowed down by extreme temperatures and this effect increased with age when slime moulds age ranged from 25 to 100 weeks. Temperature is known to contribute to senescence in most living-organisms [83], including slime moulds [71]. Susceptibility to thermal stress increases as an organism ages due to more molecular damage being generated and accumulated over time [84]. Yet, surprisingly, the effect of temperature was the strongest for the youngest slime moulds which were 8 weeks old. As our culture alternated between 25°C during the week and 12°C during the weekend, it might be possible that a thermotolerance was indirectly induced over time. Signs of habituation to low temperature has already been observed in slime moulds [85]. In *P. polycephalum* as in most living organisms, expression of heat-shock protein (HSP) increases after

1 398 exposure to a moderate thermal stress [86]. HSPs function as molecular chaperones, they reduce
2 399 molecular damage induced by extreme temperatures allowing the organism to cope with this stressor
3 400 [87–90]. Usually, how much HSPs an organism produces correlates with the level of stress to which it
4 401 is exposed. Therefore, in our experiment, we can presume that the level of expression of HSPs was
5 402 higher in older slime moulds than in younger ones as they experienced more periodic changes in
6 403 temperature over time. Thus, older slime moulds might have been more susceptible to thermal stress
7 404 owing to their age but also more thermotolerant due to their past experiences than younger slime
8 405 moulds.

15 406 Recently [74,91], it was revealed that slime moulds are capable of habituation, a simple form
16 407 of learning, when they are repeatedly exposed to a chemical stimulus. In animals, learning performance
17 408 displays a progressive decrease with age. However when it comes to habituation, it was observed that
18 409 older organisms habituate more rapidly than young ones in *Aplysia californica* [92] and *Caenorhabditis*
19 410 *elegans* [93] or as fast as young ones in *Drosophila melanogaster* [94], mice [95] and rats [96]. Here,
20 411 we showed that, habituation performance is preserved in old slime moulds as well. In animals, it was
21 412 shown that the response threshold to the stimulus was higher in old animals than in young ones which
22 413 could explain their performance [92,95]. In contrast, in our experiment, we showed that the aversion
23 414 level toward NaCl did not vary with age. On the contrary, in our first experiment, older individuals
24 415 seemed more susceptible to NaCl than younger ones. Habituation to NaCl in slime moulds relies, in
25 416 part, on NaCl uptake [41]. In plants, yeast and cellular slime moulds, sodium is driven into the cell
26 417 passively through non selective cationic channels [97–99]. Therefore, it is possible that this passive
27 418 transport of sodium throughout the membrane is not affected by age.

37 419 In slime moulds, sclerotia have been found to survive under adverse environmental conditions
38 420 for long periods up to 3 years. In the slime mould *Didymimim iridis*, a related species, the aging process
39 421 stops when the plasmodium differentiates into a sclerotium [100]. Once the sclerotium is revived, the
40 422 plasmodium then lives the remainder of its characteristic life spans [100]. In contrast in this study, we
41 423 observed a recovery phenomenon when aged slime moulds went throughout a dormant stage. After
42 424 dormancy, old slime moulds migrated as fast as young slime moulds and faster than old slime moulds
43 425 which had remained in a plasmodial stage. Past works have shown that oxidative stress defences are
44 426 involved in the transition from plasmodium to sclerotium in *P. polycephalum* [101,102]. However,
45 427 cell senescence is in part due to oxidative damage to DNA, RNA and proteins by mitochondrial reactive
46 428 oxygen species (ROS) which accumulate with age [103]. Hence, one hypothesis might be that oxidative
47 429 stress defences expressed during differentiation were responsible for the elimination of harmful ROS
48 430 and, as a consequence, the behavioural recovery observed in old slime molds after dormancy.
49 431 However, we only recorded the behaviour of the slime moulds one week after they were reinitiated
50 432 from sclerotia and we cannot rule out that this rejuvenation phenomenon was only transient and did
51 433 not affect the plasmodium lifespan.

1 434 In slime moulds, cell fusion is extremely common. In a related species *Didymium nigripes*, it
2 435 was shown that slime moulds lose the ability to fuse over time [104]. In this study, in contrast, we
3 436 showed that fusion occurred regardless of slime mould age. As old slime moulds were slower than their
4 437 younger counterparts, their latency to fuse was simply longer. Vein fusion took also a longer time in
5 438 old slime moulds than in young ones, but fusion was never aborted. We also revealed that an old slime
6 439 mould could recover its migration speed performance after fusing with a young counterpart. Resource
7 440 sharing between cells is often observed as a strategy to cope with environmental stress. For instance,
8 441 cells can exchange learned information [25], mitochondria [105] or membrane material [106] via cell
9 442 fusion. It is well known that cells accumulate DNA, protein and metabolite damage over time [107]. In
10 443 young cells, most of the damages are cleared by maintenance processes [107]. However, as not all
11 444 damages can be repaired, irreparable damage rises in the cell through time [107]. In aged cells, the
12 445 maintenance systems can be damaged themselves, amplifying the burden of damages [107]. In our
13 446 experiment, it might be possible that the damages accumulated over time in an old slime mould were
14 447 cleaned or repaired by the maintenance system of its younger counterpart. Interestingly, in a related
15 448 species, *Didymium iridis*, when age-hybrid slime moulds were formed by the fusion of young and old
16 449 individuals, 58 % of the new slime moulds acquired the remaining life span of the older individuals,
17 450 22 % died concurrently with the younger individuals and 20% died at an intermediate age [108]. The
18 451 authors concluded that it is the oldest slime mould that determines the lifespan of the age-hybrid slime
19 452 mould [100,109]. In our experiment, we did not follow the age-hybrid slime moulds behaviour over
20 453 time. Hence, even if we observed a form of recovery process in old slime moulds after fusion, we
21 454 cannot rule out that they would have died concurrently with slime moulds of the same age in absence of
22 455 fusion.

23 456 As *P. polycephalum* migrates on the substrate, it leaves behind an extracellular slime [42,110].
24 457 Previous works have shown that previously explored substrate might be repulsive or attractive
25 458 depending on the physiological status of the slime mould that explored the substrate [21,44]. A
26 459 substrate explored by a starved, irradiated or poisoned slime mould is actively avoided [44] while a
27 460 substrate explored by a well-fed slime mould is attractive [21]. Calcium has been identified as the main
28 461 chemical that mediates attraction [21] whereas the substances responsible for the aversion remain to
29 462 be identified. In this study, we showed that slime moulds were able to use age-related cues released by
30 463 conspecifics and make a decision accordingly. We revealed that slime moulds, regardless of their age,
31 464 were more attracted by cues released by younger slime moulds than by older ones. As we did not notice
32 465 any fusion delay when a young slime mould was facing an old one in the fusion experiment, we believe
33 466 that the substrate explored by an old slime mould was not aversive but only less attractive. The
34 467 information supplied by these excretions would appear to be crucial for both young and old slime
35 468 mould. A young slime mould would benefit from avoiding fusion with an aged slime mould as it could
36 469 inherit its damages. Indeed, we noticed that after fusing with an old counterpart, a young slime mould

1 470 was somewhat slower. In contrast, an old slime mould would gain from fusing with a young slime
 2 471 mould, as it would allow it to recover. **In many animals from flies to humans [111–115], as an organism**
 3 472 **ages, the effect of senescence on both the perception and processing of information might affect their**
 4 473 **response to the presence of others. Here, we did not observe any effect of age on social interactions.**
 5 474 **since older individuals were still able to fuse with others (although in a slower fashion) and detect**
 6 475 **conspecifics.**

7 476 Although many studies have studied behaviour in single cell organisms, few have taken the
 8 477 step of looking for changes in behaviour over the lifetime of individuals. This study extends our
 9 478 knowledge of the behavioural plasticity of unicellular organisms and establish acellular slime molds as
 10 479 a promising model to investigate the effect of aging on behaviour at the cellular level. Their ability to
 11 480 fuse with congeners and their peculiar lifecycle, might help us to understand how cellular metabolic
 12 481 activity and cellular defences are regulated and coordinated, and how a cell can counteract the effects
 13 482 of ageing.
 14 483

15 484 References

- 16 485 1. Waters CM, Bassler BL. 2005 Quorum sensing: cell-to-cell communication in bacteria. *Annu. Rev.*
 17 486 *Cell Dev. Biol.* **21**, 319–346. (doi:10.1146/annurev.cellbio.21.012704.131001)
- 18 487 2. Whiteley M, Diggle SP, Greenberg EP. 2017 Progress in and promise of bacterial quorum sensing
 19 488 research. *Nature* **551**, 313–320. (doi:10.1038/nature24624)
- 20 489 3. Gross JD, Peacey MJ, Trevan DJ. 1976 Signal emission and signal propagation during early
 21 490 aggregation in *Dictyostelium discoideum*. *J. Cell Sci.* **22**, 645–656.
- 22 491 4. Bonner JT. 1959 „The cellular Slime moulds” Princeton Univ. Press. New Jersey)
- 23 492 5. Sawai S, Thomason PA, Cox EC. 2005 An autoregulatory circuit for long-range self-organization in
 24 493 *Dictyostelium* cell populations. *Nature* **433**, 323–326.
- 25 494 6. Gregor T, Fujimoto K, Masaki N, Sawai S. 2010 The onset of collective behavior in social amoebae.
 26 495 *Science (80-.).* **328**, 1021–1025.
- 27 496 7. Bell G. 1984 Evolutionary and nonevolutionary theories of senescence. *Am. Nat.* **124**, 600–603.
- 28 497 8. Kirkwood TBL, Austad SN. 2000 Why do we age? *Nature* **408**, 233–238.
- 29 498 9. Moger-Reischer RZ, Lennon JT. 2019 Microbial ageing and longevity. *Nat. Rev. Microbiol.* **17**, 679–
 30 499 690. (doi:10.1038/s41579-019-0253-y)
- 31 500 10. Ackermann M, Chao L, Bergstrom CT, Doebeli M. 2007 On the evolutionary origin of aging. *Aging*
 32 501 *Cell* **6**, 235–244.
- 33 502 11. Łapińska U, Glover G, Capilla-Lasheras P, Young AJ, Pagliara S. 2019 Bacterial ageing in the
 34 503 absence of external stressors. *Philos. Trans. R. Soc. B* **374**, 20180442.
- 35 504 12. Florea M. 2017 Aging and immortality in unicellular species. *Mech. Ageing Dev.* **167**, 5–15.
 36 505 (doi:https://doi.org/10.1016/j.mad.2017.08.006)

- 1 506 13. Crane MM, Kaeberlein M. 2018 The paths of mortality: How understanding the biology of aging
2 507 can help explain systems behavior of single cells. *Curr. Opin. Syst. Biol.* **8**, 25–31.
3 508 (doi:https://doi.org/10.1016/j.coisb.2017.11.010)
4 509 14. Reid CR, Latty T. 2016 Collective behaviour and swarm intelligence in slime moulds. *FEMS*
5 510 *Microbiol. Rev.* , fuw033. (doi:10.1093/femsre/fuw033)
6 511 15. Takamatsu A, Fujii T, Endo I. 2000 Time delay effect in a living coupled oscillator system with the
7 512 plasmodium of *Physarum polycephalum*. *Phys. Rev. Lett.* **85**, 2026.
8 513 16. Boussard A, Fessel A, Oettmeier C, Briard L, Döbereiner H-G, Dussutour A. 2020 Adaptive
9 514 behavior and learning in slime moulds: the role of oscillations. *Philos. Trans. R. Soc. London. B*
10 515 **376**, 20190757.
11 516 17. Yoshiyama S, Ishigami M, Nakamura A, Kohama K. 2010 Calcium wave for cytoplasmic streaming
12 517 of *Physarum polycephalum*. *Cell Biol. Int.* **34**, 35–40.
13 518 18. Alim K, Amselem G, Peaudecerf F, Brenner MP, Pringle A. 2013 Random network peristalsis in
14 519 *Physarum polycephalum* organizes fluid flows across an individual. *Proc. Natl. Acad. Sci.* **110**,
15 520 13306–13311.
16 521 19. Meyer B, Ansorge C, Nakagaki T. 2017 The role of noise in self-organized decision making by the
17 522 true slime mold *Physarum polycephalum*. *PLoS One* **12**, e0172933.
18 523 20. Vogel D, Dussutour A, Deneubourg J-L. 2018 Symmetry breaking and inter-clonal behavioural
19 524 variability in a slime mould. *Biol. Lett.* **14**, 20180504.
20 525 21. Vogel D, Nicolis SC, Perez-Escudero A, Nanjundiah V, Sumpter DJT, Dussutour A. 2015 Phenotypic
21 526 variability in unicellular organisms: from calcium signalling to social behaviour. *Proc. R. Soc. B*
22 527 *Biol. Sci.* **282**, 20152322.
23 528 22. Dussutour A, Ma Q, Sumpter D. 2019 Phenotypic variability predicts decision accuracy in
24 529 unicellular organisms. *Proc. R. Soc. B* **286**, 20182825.
25 530 23. Zabzina N, Dussutour A, Mann RP, Sumpter DJT, Nicolis SC. 2014 Symmetry restoring bifurcation
26 531 in collective decision-making. *PLoS Comput. Biol.* **10**, e1003960.
27 532 24. Fessel A, Oettmeier C, Bernitt E, Gauthier NC, Döbereiner H-G. 2012 *Physarum polycephalum*
28 533 Percolation as a Paradigm for Topological Phase Transitions in Transportation Networks. *Phys.*
29 534 *Rev. Lett.* **109**. (doi:10.1103/PhysRevLett.109.078103)
30 535 25. Vogel D, Dussutour A. 2016 Direct transfer of learned behaviour via cell fusion in non-neural
31 536 organisms. *Proc. R. Soc. B Biol. Sci.* **283**, 20162382.
32 537 26. Ray SK, Valentini G, Shah P, Haque A, Reid CR, Weber GF, Garnier S. 2019 Information transfer
33 538 during food choice in the slime mold *Physarum polycephalum*. *Front. Ecol. Evol.* **7**, 67.
34 539 27. Reid CR, Garnier S, Beekman M, Latty T. 2015 Information integration and multiattribute decision
35 540 making in non-neuronal organisms. *Anim. Behav.* **100**, 44–50.
36 541 (doi:10.1016/j.anbehav.2014.11.010)
37 542 28. Reid CR, MacDonald H, Mann RP, Marshall JAR, Latty T, Garnier S. 2016 Decision-making without
38 543 a brain: how an amoeboid organism solves the two-armed bandit. *J. R. Soc. Interface* **13**,
39 544 20160030.

- 1 545 29. Beekman M, Latty T. 2015 Brainless but Multi-Headed: Decision Making by the Acellular Slime
2 Mould *Physarum polycephalum*. *J. Mol. Biol.* **427**, 3734–3743. (doi:10.1016/j.jmb.2015.07.007)
- 3 546
- 4 547 30. Nakagaki T, Kobayashi R, Nishiura Y, Ueda T. 2004 Obtaining multiple separate food sources:
5 behavioural intelligence in the *Physarum plasmodium*. *Proc. R. Soc. London. Ser. B Biol. Sci.* **271**,
6 548 2305–2310. (doi:10.1098/rspb.2004.2856)
- 7 549
- 8 550 31. Jones J. 2015 ECC 15 - From Pattern Formation to Material Computation. , 1–369.
- 9 551 32. Gao C, Liu C, Schenz D, Li X, Zhang Z, Jusup M, Wang Z, Beekman M, Nakagaki T. 2019 Does being
10 multi-headed make you better at solving problems? A survey of *Physarum*-based models and
11 552 computations. *Phys. Life Rev.* **29**, 1–26.
- 12 553
- 13 554 33. Nakagaki T, Yamada H, Tóth Á. 2000 Maze-solving by an amoeboid organism. *Nature* **407**, 470.
- 14 555 34. Tero A, Takagi S, Saigusa T, Ito K, Bebbler DP, Fricker MD, Yumiki K, Kobayashi R, Nakagaki T.
15 2010 Rules for biologically inspired adaptive network design. *Science (80-.)*. **327**, 439–442.
- 16 556
- 17 557 35. Oettmeier C, Nakagaki T, Döbereiner H-G. 2020 Slime mold on the rise: The physics of *Physarum*
18 polycephalum. *J. Phys. D. Appl. Phys.* **53**, 310201.
- 19 558
- 20 559 36. Awad A, Pang W, Lusseau D, Coghill GM. 2021 A Survey on *Physarum Polycephalum* Intelligent
21 Foraging Behaviour and Bio-Inspired Applications.
- 22 560
- 23 561 37. Oettmeier C, Brix K, Döbereiner H-G. 2017 *Physarum polycephalum*-a new take on a classic
24 model system. *J. Phys. D. Appl. Phys.* **50**, 1–12.
- 25 562
- 26 563 38. Reid CR, Beekman M. 2013 Solving the Towers of Hanoi--how an amoeboid organism efficiently
27 constructs transport networks. *J. Exp. Biol.* **216**, 1546–1551.
- 28 564
- 29 565 39. Saigusa T, Tero A, Nakagaki T, Kuramoto Y. 2008 Amoebae Anticipate Periodic Events. *Phys. Rev.*
30 *Lett.* **100**. (doi:10.1103/PhysRevLett.100.018101)
- 31 566
- 32 567 40. Boisseau RP, Vogel D, Dussutour A. 2016 Habituation in non-neural organisms: evidence from
33 slime moulds. *Proc. R. Soc. B Biol. Sci.* **283**, 20160446. (doi:10.1073/pnas.78.8.5245)
- 34 568
- 35 569 41. Boussard A, Delescluse J, Pérez-Escudero A, Dussutour A. 2019 Memory inception and
36 preservation in slime moulds: the quest for a common mechanism. *Philos. Trans. R. Soc. B* **374**,
37 570 20180368.
- 38 571
- 39 572 42. Reid CR, Latty T, Dussutour A, Beekman M. 2012 Slime mold uses an externalized spatial
40 “memory” to navigate in complex environments. *Proc. Natl. Acad. Sci.* **109**, 17490–17494.
- 41 573
- 42 574 43. Kramar M, Alim K. 2021 Encoding memory in tube diameter hierarchy of living flow network.
43 *Proc. Natl. Acad. Sci.* **118**, e2007815118.
- 44 575
- 45 576 44. Briard L, Gourjade C, Bousquet C, Dussutour A. 2020 Stress signalling in acellular slime moulds
46 and its detection by conspecifics. *Philos. Trans. R. Soc. B* **375**, 20190470.
- 47 577
- 48 578 45. Dussutour A, Latty T, Beekman M, Simpson SJ. 2010 Amoeboid organism solves complex
49 nutritional challenges. *Proc. Natl. Acad. Sci.* **107**, 4607–4611. (doi:10.1073/pnas.0912198107)
- 50 579
- 51 580 46. Murugan NJ *et al.* 2021 Mechanosensation Mediates Long-Range Spatial Decision-Making in an
52 Aneural Organism. *Adv. Mater.* **33**. (doi:10.1002/adma.202008161)
- 53 581
- 54 582 47. Reid CR, Garnier S, Beekman M, Latty T. 2015 Information integration and multiattribute decision
55 making in non-neuronal organisms. *Anim. Behav.* **100**, 44–50.
- 56 583

- 1 584 48. Baumgarten W, Hauser MJB. 2013 Functional organization of the vascular network of *Physarum*
2 polycephalum. *Phys. Biol.* **10**. (doi:10.1088/1478-3975/10/2/026003)
- 3 585
- 4 586 49. Matsumoto K, Takagi S, Nakagaki T. 2008 Locomotive mechanism of *Physarum* plasmodia based
5 on spatiotemporal analysis of protoplasmic streaming. *Biophys. J.* **94**, 2492–2504.
- 6 587
- 7 588 50. Lewis OL, Zhang S, Guy RD, Del Alamo JC. 2015 Coordination of contractility, adhesion and flow in
8 migrating *Physarum* amoebae. *J. R. Soc. Interface* **12**, 20141359.
- 9 589
- 10 590 51. Baumgarten W, Hauser MJB. 2014 Dynamics of frontal extension of an amoeboid cell. *EPL*
11 (Europhysics Lett. **108**, 50010. (doi:10.1209/0295-5075/108/50010)
- 12 591
- 13 592 52. Durham AC, Ridgway EB. 1976 Control of chemotaxis in *Physarum* polycephalum. *J. Cell Biol.* **69**,
14 218–223.
- 15 593
- 16 594 53. Miyake Y, Tada H, Yano M, Shimizu H. 1994 Relationship between intracellular period
17 modulation and external environment change in *Physarum* plasmodium. *Cell Struct. Funct.* **19**,
18 363–370.
- 19 595
- 20 596
- 21 597 54. Matsumoto K, Ueda T, Kobatake Y. 1986 Propagation of phase wave in relation to tactic
22 responses by the plasmodium of *Physarum* polycephalum. *J. Theor. Biol.* **122**, 339–345.
- 23 598
- 24 599 55. Marwan W. 2001 Chapter 20 Photomovement and photomorphogenesis in *Physarum*
25 polycephalum: targeting of cytoskeleton and gene expression by light. *Compr. Ser. Photosciences*
26 **1**, 561–587. (doi:10.1016/S1568-461X(01)80024-7)
- 27 600
- 28 601
- 29 602 56. Wolf R, Niemuth J, Sauer H. 1997 Thermotaxis and protoplasmic oscillations in *Physarum*
30 plasmodia analysed in a novel device generating stable linear temperature gradients.
31 *Protoplasma* **197**, 121–131.
- 32 603
- 33 604
- 34 605 57. Wohlfarth-Bottermann KE. 1977 Oscillating contractions in protoplasmic strands of *Physarum*:
35 simultaneous tensiometry of longitudinal and radial rhythms, periodicity analysis and
36 temperature dependence. *J. Exp. Biol.* **67**, 49–59. (doi:10.1242/jeb.67.1.49)
- 37 606
- 38 607
- 39 608 58. Halvorsrud R, Laane MM, Giaever I. 1995 A Novel Electrical Method to Study Plasmodial
40 Contractions in *Physarum*. Synchrony and Temperature Dependence. *Biol. Rhythm Res.* **26**, 316–
41 330. (doi:10.1080/09291019509360345)
- 42 609
- 43 610
- 44 611 59. Block I, Wolke A, Briegleb W. 1994 Gravitational response of the slime mold *Physarum*. *Adv. Sp.*
45 *Res.* **14**, 21–34.
- 46 612
- 47 613 60. Aldrich H. 2012 *Cell biology of Physarum and Didymium V1: organisms, nucleus, and cell cycle*.
48 Elsevier.
- 49 614
- 50 615 61. Rojas C, Stephenson SL. 2021 *Myxomycetes: biology, systematics, biogeography and ecology*.
51 Academic Press.
- 52 616
- 53 617 62. Poulter RTM. 1969 Senescence in the Myxomycete *Physarum* polycephalum.
- 54 618
- 55 619 63. Nakagawa CC, Jones EP, Miller DL. 1998 Mitochondrial DNA rearrangements associated with mF
56 plasmid integration and plasmodial longevity in *Physarum* polycephalum. *Curr. Genet.* **33**, 178–
57 187. (doi:10.1007/s002940050325)
- 58 620
- 59 621 64. Hu FS, Clark J, Lott T. 1985 Recurrent senescence in axenic cultures of *Physarum* polycephalum. *J.*
60 *Gen. Microbiol.* **131**, 811–815. (doi:10.1099/00221287-131-4-811)
- 622

- 1 623 65. Lott T, Clark J. 1980 Plasmodial senescence in the acellular slime mold *Didymium iridis*. *Exp. Cell*
2 624 *Res.* **128**, 455–457. (doi:10.1016/0014-4827(80)90080-4)
- 3 625 66. Adamatzky A. 2017 Thirty seven things to do with live slime mould. In *Advances in*
4 626 *Unconventional Computing*, pp. 709–738. Springer.
- 5 627 67. Keller HW, Everhart SE. 2010 Importance of Myxomycetes in Biological Research and Teaching.
6 628 *Society* **3**, 13–27.
- 7 629 68. Abe T, Takano H, Sasaki N, Mori K, Kawano S. 2000 In vitro DNA fragmentation of mitochondrial
8 630 DNA caused by single-stranded breakage related to macroplasmodial senescence of the true
9 631 slime mold, *Physarum polycephalum*. *Curr. Genet.* **37**, 125–135. (doi:10.1007/s002940050019)
- 10 632 69. Adler PN, Holt CE. 1974 Change in properties of *Physarum polycephalum* amoebae during
11 633 extended culture. *J. Bacteriol.* **120**, 532–533. (doi:10.1128/jb.120.1.532-533.1974)
- 12 634 70. MCCULLOUGH CHR, Cooke DJ, Foxon JL, SUDBERY PE, GRANT WD. 1973 Nuclear DNA content
13 635 and senescence in *Physarum polycephalum*. *Nat. New Biol.* **245**, 263–265.
- 14 636 71. Clark J, Lott T. 1981 Aging in the acellular slime mold *Didymium iridis*: Temperature and
15 637 nutritional effects. *Exp. Mycol.* **5**, 369–372. (doi:10.1016/0147-5975(81)90043-8)
- 16 638 72. Matsumoto K, Ueda T, Kobatake Y. 1988 Reversal of thigmotaxis with oscillatory stimulation in
17 639 the plasmodium of *Physarum polycephalum*. *J. Theor. Biol.* **131**, 175–182.
- 18 640 73. Latty T, Beekman M. 2010 Food quality and the risk of light exposure affect patch-choice
19 641 decisions in the slime mold *Physarum polycephalum*. *Ecology* **91**, 22–27.
- 20 642 74. Dussutour A. 2021 Learning in single cell organisms. *Biochem. Biophys. Res. Commun.* **564**, 92–
21 643 102. (doi:10.1016/j.bbrc.2021.02.018)
- 22 644 75. Bates D, Sarkar D, Bates MD, Matrix L. 2007 The lme4 package. *R Packag. version* **2**, 74.
- 23 645 76. Patino-Ramirez F, Boussard A, Arson C, Dussutour A. 2019 Substrate composition directs slime
24 646 molds behavior. *Sci. Rep.* **9**, 1–14.
- 25 647 77. Denbo JR, Miller DM. 1976 Factors affecting the movement of slime mold plasmodia. *Comp.*
26 648 *Biochem. Physiol. -- Part A Physiol.* **55**, 5–12. (doi:10.1016/0300-9629(76)90114-6)
- 27 649 78. Ueda T, Terayama K, Kurihara K, Kobatake Y. 1975 Threshold phenomena in chemoreception and
28 650 taxis in slime mold *Physarum polycephalum*. *J. Gen. Physiol.* **65**, 223–234.
- 29 651 79. Dmitrieva NI, Burg MB. 2007 High NaCl promotes cellular senescence. *Cell Cycle* **6**, 3108–3113.
- 30 652 80. Dmitrieva NI, Cui K, Kitchaev DA, Zhao K, Burg MB. 2011 DNA double-strand breaks induced by
31 653 high NaCl occur predominantly in gene deserts. *Proc. Natl. Acad. Sci.* **108**, 20796–20801.
- 32 654 81. Lomagin AG. 1978 Repair of functional and ultrastructural alterations after thermal injury of
33 655 *Physarum polycephalum*. *Planta* **142**, 123–134. (doi:10.1007/BF00388203)
- 34 656 82. Bernstam VA, Arndt S. 1974 Effects of supraoptimal temperatures on the myxomycete *Physarum*
35 657 *polycephalum*. *Arch. Microbiol.* **95**, 357–363. (doi:10.1007/bf02451777)
- 36 658 83. Keil G, Cummings E, de Magalhaes JP. 2015 Being cool: how body temperature influences ageing
37 659 and longevity. *Biogerontology* **16**, 383–397.
- 38 660 84. Conti B. 2008 Considerations on temperature, longevity and aging. *Cell. Mol. Life Sci.* **65**, 1626–
39 661 1630.

- 1 662 85. Shirakawa T, Gunji Y-P, Miyake Y. 2011 An associative learning experiment using the
2 663 plasmodium of *Physarum polycephalum*. *Nano Commun. Netw.* **2**, 99–105.
- 3 664 86. Gevers M, Fracella F, Rensing L. 2006 Nuclear translocation of constitutive heat shock protein 70
4 665 during S phase in synchronous macroplasmodia of *Physarum polycephalum*. *FEMS Microbiol.*
5 666 *Lett.* **152**, 89–94. (doi:10.1111/j.1574-6968.1997.tb10413.x)
- 6 667 87. Lindquist S. 1986 The heat-shock response. *Annu. Rev. Biochem.* **55**, 1151–1191.
- 7 668 88. Kregel KC. 2002 Invited review: heat shock proteins: modifying factors in physiological stress
8 669 responses and acquired thermotolerance. *J. Appl. Physiol.* **92**, 2177–2186.
- 9 670 89. Sørensen JG, Kristensen TN, Loeschcke V. 2003 The evolutionary and ecological role of heat shock
10 671 proteins. *Ecol. Lett.* **6**, 1025–1037.
- 11 672 90. Chen B, Feder ME, Kang L. 2018 Evolution of heat-shock protein expression underlying adaptive
12 673 responses to environmental stress. *Mol. Ecol.* **27**, 3040–3054.
- 13 674 91. Boisseau RP, Vogel D, Dussutour A. 2016 Habituation in non-neural organisms: evidence from
14 675 slime moulds. *Proc. R. Soc. B Biol. Sci.* **283**, 20160446.
- 15 676 92. Rattan KS, Peretz B. 1981 Age-dependent behavioral changes and physiological changes in
16 677 identified neurons in *Aplysia californica*. *J. Neurobiol.* **12**, 469–478.
- 17 678 93. Beck CDO, Rankin CH. 1993 Effects of aging on habituation in the nematode *Caenorhabditis*
18 679 *elegans*. *Behav. Processes* **28**, 145–163.
- 19 680 94. Le Bourg E. 1983 Aging and habituation of the tarsal response in *Drosophila melanogaster*.
20 681 *Gerontology* **29**, 388–393.
- 21 682 95. Brennan MJ, Allen D, Aleman D, Azmitia EC, Quartermain D. 1984 Age differences in within-
22 683 session habituation of exploratory behavior: effects of stimulus complexity. *Behav. Neural Biol.*
23 684 **42**, 61–72.
- 24 685 96. Shukitt-Hale B, Casadesus G, Cantuti-Castelvetri I, Joseph JA. 2001 Effect of age on object
25 686 exploration, habituation, and response to spatial and nonspatial change. *Behav. Neurosci.* **115**,
26 687 1059.
- 27 688 97. De Hertogh B, Hancy F, Goffeau A, Baret P V. 2006 Emergence of species-specific transporters
28 689 during evolution of the hemiascomycete phylum. *Genetics* **172**, 771–781.
- 29 690 98. Müller U, Hartung K. 1990 Properties of three different ion channels in the plasma membrane of
30 691 the slime mold *Dictyostelium discoideum*. *Biochim. Biophys. Acta - Biomembr.* **1026**, 204–212.
31 692 (doi:https://doi.org/10.1016/0005-2736(90)90065-V)
- 32 693 99. Demidchik V, Davenport RJ, Tester M. 2002 Nonselective cation channels in plants. *Annu. Rev.*
33 694 *Plant Biol.* **53**, 67–107.
- 34 695 100. Lott T, Clark J. 1982 Sclerotization in Relation to Plasmodial Senescence in the Acellular Slime
35 696 Mould *Didymium iridis*. *Microbiology* **128**, 1483–1487.
- 36 697 101. Schreckenbach T, Werenskiold A-K. 1986 Gene expression during plasmodial differentiation. In
37 698 *The Molecular Biology of Physarum polycephalum*, pp. 131–150. Springer.
- 38 699 102. Allen RG, Newton RK, Sohal RS, Shipley GL, Nations C. 1985 Alterations in superoxide dismutase,
39 700 glutathione, and peroxides in the plasmodial slime mold *Physarum polycephalum* during

- 1 701 differentiation. *J. Cell. Physiol.* **125**, 413–419.
- 2
- 3 702 103. López-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. 2013 The hallmarks of aging. *Cell*
- 4 703 **153**, 1194–1217.
- 5
- 6 704 104. Kerr NS, Waxlax JN. 1968 A yellow variant of the eumycetozoon *Didymium nigripes* which
- 7 705 exhibits aging. *J. Exp. Zool.* **168**, 351–361. (doi:10.1002/jez.1401680306)
- 8
- 9 706 105. Spees JL, Olson SD, Whitney MJ, Prockop DJ. 2006 Mitochondrial transfer between cells can
- 10 707 rescue aerobic respiration. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 1283–1288.
- 11 708 (doi:10.1073/pnas.0510511103)
- 12
- 13 709 106. Vassallo CN, Wall D. 2016 Tissue repair in myxobacteria: A cooperative strategy to heal cellular
- 14 710 damage. *BioEssays* **38**, 306–315. (doi:10.1002/bies.201500132)
- 15
- 16 711 107. Gladyshev VN *et al.* 2021 Molecular damage in aging. *Nat. Aging* **1**, 1096–1106.
- 17 712 108. Clark J, Hakim R. 1980 Aging of plasmodial heterokaryons in *Didymium iridis*. *Mol. Gen. Genet.*
- 18 713 *MGG* **178**, 419–422.
- 19 714 109. Clark J, Lott T. 1989 Age heterokaryon studies in *Didymium iridis*. *Mycologia* **81**, 636–638.
- 20 715 110. Reid CR, Beekman M, Latty T, Dussutour A. 2013 Amoeboid organism uses extracellular
- 21 716 secretions to make smart foraging decisions. *Behav. Ecol.* **24**, 812–818.
- 22 717 111. Almeling L, Hammerschmidt K, Sennhenn-Reulen H, Freund AM, Fischer J. 2016 Motivational
- 23 718 shifts in aging monkeys and the origins of social selectivity. *Curr. Biol.* **26**, 1744–1749.
- 24 719 112. Brenman-Suttner DB, Yost RT, Frame AK, Robinson JW, Moehring AJ, Simon AF. 2020 Social
- 25 720 behavior and aging: A fly model. *Genes, Brain Behav.* **19**, e12598.
- 26 721 113. Rosati AG, Hagberg L, Enigk DK, Otali E, Emery Thompson M, Muller MN, Wrangham RW,
- 27 722 Machanda ZP. 2020 Social selectivity in aging wild chimpanzees. *Science (80-)*. **370**, 473–476.
- 28 723 114. Charles ST, Carstensen LL. 2010 Social and emotional aging. *Annu. Rev. Psychol.* **61**, 383–409.
- 29 724 115. Siracusa ER, Higham JP, Snyder-Mackler N, Brent LJN. 2022 Social ageing: exploring the drivers of
- 30 725 late-life changes in social behaviour in mammals. *Biol. Lett.* **18**, 20210643.
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8 730 **Data Accessibility**

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10 732 The datasets supporting this article are provided as supplementary material.

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16 735 **Authors' Contributions**

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18 737 AD conceived the study. AD designed the study. AR, EP, PM, CC and MD performed the experiments. AR, EP, PM and CC carried out

19 738 data acquisition. AD analysed the data. AD wrote the manuscript. AD secured funding. All authors gave final approval for publication.

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24 740 **Competing Interests**

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26 742 We have no competing interests.

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3 749 **Figure 1:** Experimental design. (a) Effect of various substrate (nutritive substrate: 5% oat gel, aversive
4 substrates: 0.6% NaCl + oat gel, 0.8% NaNO₃ + oat gel) on migration speed experiment as a function
5 750 of slime mould age (6 groups of different age were tested, from 1 to 94 weeks old). (b) Effect of
6 751 temperature (standard temperature : 25°C, extreme temperature : 10°C and 38.5°C) on migration speed
7 752 as a function of slime mould age (5 groups of different age were tested from 8 to 101 weeks old). (c)
8 753 Decision making under nutritive (2.5% oat gel vs 5% oat gel) and aversive conditions (0.4% NaCl +
9 754 5% oat gel vs 0.6% NaCl + 5% oat gel, 0.4% NaNO₃ + 5% oat gel vs 0.6% NaNO₃ + 5% oat gel) as a
10 755 function of slime mould age (5 groups of different age were tested, from 6 to 99 weeks old). (d)
11 756 Habituation to an aversive substance (0.6% NaCl) as a function of slime mould age (2 groups of
12 757 different age were tested : young slime moulds of 12 weeks and old ones of 74 weeks). (e) Effect of
13 758 dormancy on migration speed on a neutral (agar gel) and nutritive substrate (oat gel) as a function of
14 759 slime mould age (2 groups of different age were tested : old slime mould of 60 weeks and very old
15 760 ones of 100 weeks). (f) Fusion ability as a function of slime mould age (2 groups of different age were
16 761 tested: : relatively young slime moulds of 18 weeks and old ones of 75 weeks old). (g) Effect of fusion
17 762 on migration speed on a nutritive substrate (oat gel) as a function of slime mould age (2 groups of
18 763 different age were tested: young slime mould of 5 weeks and old ones of 54 weeks). (h) Detection of
19 764 chemical cues left by conspecifics (young and old) as a function of slime mould age (2 groups of
20 765 different ages were tested: young slime moulds of 3 weeks and old ones of 52 weeks).

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24 769 **Figure 2:** Mean migration speed as a function of age and substrate. Six groups of 10 large plasmodia
25 770 of different ages (1, 17, 32, 54, 74 and 94 weeks old) were used to conduct this experiment. The
26 771 substrates were made of oat gel (5%w/v) containing one of two aversive substances (Oat + NaCl (0.6%
27 772 w/v), Oat + NaNO₃ (0.8% w/v)) or not (Oat). N > 48 for each age and each substrate. Total numbers
28 773 of assays= 2396. Error bars indicate CI95.

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31 776 **Figure 3:** Mean migration speed and mean proportion of motionless slime moulds as a function of age
32 777 and temperature. Five groups of 10 large plasmodia of different ages (8, 24, 61, 81 and 101 weeks old)
33 778 were used to conduct this experiment. Three temperatures were tested: a standard one (25°C) and two
34 779 extremes (10°C and 38.5°C). The substrates were made of oat gel (5% w/v). All the slime moulds at
35 780 25°C migrated on the bridge so the proportion of motionless slime mould was equal to zero for all age
36 781 groups. N > 80 for each age and each temperature. Total numbers of assays= 3533. Error bars indicate
37 782 CI95.

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3 785 **Figure 4:** Decision making as a function of age and substrates. Five groups of 10 large plasmodia of
4 786 different ages (6, 22, 59, 79 and 99 weeks old) were used to conduct this experiment. Under non
5 787 aversive conditions (oat), the slime moulds were offered a choice between a high-quality oat gel (5%
6 788 w/v) and a low-quality one (2.5% w/v). Under aversive conditions (Oat+ NaCl or Oat+NaNO₃), the
7 789 slime moulds were given a choice between a high-aversive option (0.6% w/v) and a low-aversive
8 790 option (0.4% w/v). Decision making performance was computed as the distance covered on the best
9 791 bridge divided by the total distance. A value equal to 0.5 means that the slime mould distribution is
10 792 symmetric between the two bridges, a value equal to 1 or 0 means that the slime moulds migrated only
11 793 one the best or the worse bridge respectively. N > 235 for each age and each condition. Total numbers
12 794 of assays= 4206. Error bars indicate CI95.

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22 796 **Figure 5:** Learning as a function of age. (a) Migration speed on day 1. Two groups of 10 large
23 797 plasmodia of two different ages 12 and 74 weeks old were used to conduct this experiment. Slime
24 798 moulds were required to migrate on an oat gel bridge with an aversive substance NaCl (Oat+ NaCl)
25 799 (0.6% w/v, habituated treatment) or a plain oat gel bridge (Oat) without the repellent (control
26 800 treatment). Low values of speed indicate an aversive response. (b) Aversion index on day 4 computed
27 801 after the habituation phase. On day 4, all slime moulds (habituated and control) had to migrate either
28 802 on a bridge with the aversive substance or without. An aversion index closes to 0 indicates habituation
29 803 while values clearly above 0 indicate an aversion to the repellent. N = 50 for each age (young and old)
30 804 and each treatment (habituated and control). Total numbers of assays= 200. Error bars indicate 95%
31 805 CI.

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42 808 **Figure 6:** Mean migration speed following a dormancy period. Two groups of 10 large plasmodia of
43 809 two different ages 60 (old) and 100 (very old) weeks old were used to conduct this experiment. Before
44 810 the migration speed assays, slime moulds were either turn into sclerotia for a week and reinitiated
45 811 (dormancy: yes) or kept as plasmodia (dormancy: no). The substrates were made of oat gel 5%w/v
46 812 (Oat) or plain agar gel 1%w/v (Agar). N =80 for each age (old and very old) and each treatment
47 813 (dormancy yes or no). Total numbers of assays= 640. Error bars indicate CI95.

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56 816 **Figure 7:** Fusion as a function of age. Two groups of 10 large plasmodia of two different ages 18
57 817 (young) and 75 (old) weeks old were used to conduct this experiment. Young (old) slime moulds were
58 818 paired with an individual of the same age or older (younger). The bridge separating the two slime
59 819 moulds within a pair was made of plain agar gel 1% w/v. (a) Latency to membrane fusion as a function

1 820 of age. The time of membrane fusion was recorded for each pair. (b) Latency to vein fusion as a
2 821 function of age. The time of vein connection following membrane fusion was recorded for each pair.
3 822 N=198, N=305 and N=202 for young/ young, young/old and old/old, respectively. Error bars indicate
4 823 CI95.
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7 826 **Figure 8:** Mean migration speed following fusion. Two groups of 10 large plasmodia of two different
8 827 ages 5 (young) and 54 (very old) weeks old were used to conduct this experiment. Before the migration
9 828 speed assays, slime moulds were allowed to fuse either with a slime mould of the same age or with a
10 829 slime mould of a different age. The substrates were made of oat gel 5%w/v (Oat). N =100 for each par
11 830 type (young/young, young/old, old/old). Total numbers of assays= 600. Error bars indicate CI95.
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14 833 **Figure 9:** Detection of conspecifics as a function of age. Two groups of 10 large plasmodia of two
15 834 different ages 3 (young) and 52 (old) weeks old were used to conduct this experiment. Slime moulds
16 835 were offered a choice between two substrates previously explored by individuals of different ages. We
17 836 computed the proportion of slime moulds that reached each substrate first. A value equal to 0.5 means
18 837 that the slime moulds contacted the substrate explored by a young individual as often as the substrate
19 838 explored by a young individual (random choice). N = 300 for each age. The CI of the random choice
20 839 are delineated by shaded areas. Error bars indicate CI95.
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