

Detect thy family: mechanisms, ecology and agricultural aspects of kin recognition in plants

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Abstract

The phenomenon that organisms can distinguish genetically related individuals from strangers (i.e. kin recognition) and exhibit more cooperative behaviors towards their relatives has been documented in a wide variety of organisms. But its occurrence in plants has only been recently considered. What emerges is that, while concerns remain about some methodologies used to document kin recognition, there is sufficient evidence to state that it exists in plants. Effects of kin recognition go well beyond reducing resource competition between related plants, and involve interactions with pollinators, pests and diseases as well as symbionts (mycorrhizal networks). It thus likely has important implications for diversity of plant populations, ecological networks and community structure. Such effects need to be further explored. Moreover, as kin selection may result in less competitive traits and thus greater population performance, it also holds promise for crop breeding. However, one would need to consider that (i) growing crops of strongly related plants will evidently forego advantages of crop diversification and (ii) outcomes of kin recognition tend to depend on environmental conditions. Therefore, the primary questions that need to be answered are: when, where and by how much kin recognition improves population performance.

Keywords: Crop breeding, Kin selection, Plant communication, Roots, Volatiles

1 Introduction

Plants often grow in dense vegetation stands, such as grasslands, woodlands, forests, but also agricultural crops, where they interact with neighbors. These interactions can be in the form of, e.g. resource competition, reproduction, facilitative habitat modification or communication. This entails that effects of a given set of traits for an individual cannot be viewed independent of the characteristics of its neighbors (e.g. Parker and Maynard Smith 1990). These plant-plant interactions in turn also play a key role in driving ecosystem processes such as carbon uptake, water- and nutrient balances, interactions with other non-plant organisms as well as crop yields (see review Anten & During 2011).

Much plant ecological research is built on the concept of individual selection, that is, plant traits of a given individual are (at least implicitly) viewed from the perspective of how these traits directly benefit the fitness of that individual. None withstanding, cooperative behavior (i.e. actions that benefit the group rather than the individual), even altruistic behavior (i.e. actions that benefit other individuals at a cost to the actor), are quite common in nature. To explain the evolution of such “non-selfish” behavior, Hamilton (1964) introduced the idea of “kin selection”. The basic concept is that if one individual helps another with whom it shares a certain number of genes, those genes will be passed through to the next generation through the enhanced fitness of that recipient individual. An important prerequisite for kinship-dependent behavior to occur, in turn, is kin recognition, the ability to detect the level of relatedness in another individual.

Kin recognition has been demonstrated in a wide variety of organisms including primates (Henkel and Setchell 2018), social insects (Nonacs 2011), fungi (Ågren et al. 2016), amoeba (Gruendheit et al. 2017) and bacteria (Rendueles et al. 2015). But the idea that it may occur in plants was long considered outlandish, the notion being that plants lack the nervous system which enables animals to recognize kin. It is well established that plants can sense and

respond to the presence of other plants, e.g. through light signals (Pierik and de Wit 2014) and physical contact (de Wit et al. 2012), or even to the status of those plants, e.g. whether or not a neighbor is being attacked by an herbivore being conveyed through volatiles (Karban et al. 2014) or stressed by drought being conveyed through sounds (Mishra et al. 2016). The fact that plants can distinguish between self- and non-self, and thus some level of identity recognition, has also been evident from the fact that many species prevent self-pollination (Fuji et al. 2016). More recent research indicates that plants may also be able to distinguish between their own roots and those of a non-self neighbor (see Chen et al. 2012 for review) and between their own herbivore-induced volatiles and those produced by another plant (Karban & Shiojiri 2009). The first evidence of kin recognition in plants came from a study on the annual *Cakile edentula* (Dudley & File 2007) finding that plants produced less roots when interacting with offspring from the same mother than with offspring from a different mother. A series of findings since then has strengthened the idea that kin recognition and associated kin discrimination (i.e. one acts differently towards kin than non-kin) occurs in plants (Fig. 1), though there is a heavy debate about methodology used. Kin discrimination has now been studied for different traits and functions in a wide variety of wild as well as domesticated plants.

The existence of kin recognition in plants has important consequences for plant ecology and evolutionary biology that go far beyond the fact that it is interesting to know that plants can recognize their family members. First, kin selection may affect the genetic structure and diversity of a population, as it tends to favor lower genetic diversity at the group level (Platt & Bever 2009). Second, cooperative traits tend to lead to greater population level performance (Anten & During 2011) and thus may have important implications for ecosystem functioning. This in turn may also have implications for agriculture (Murphy et al. 2017), since farmers tend to aim for group performance (high yields or resource-use efficiency at the crop stand

level) rather than individual performance, existence of kin discrimination could contribute to better crop performance and could thus be a breeding target (Denison 2011; Anten & Vermeulen 2016).

The objective of this paper is therefore first to critically appraise the evidence for kin discrimination and underlying mechanisms of kin recognition in plants and second to discuss implications for ecology and especially agriculture. While the degree of relatedness/kinship can range from the same genetically identical clone to different phylogenetic clades, we mainly focus on kin recognition at intraspecific level. We first give a brief introduction to kin selection and related concepts and describe under what conditions it is most likely to occur. We then explore the evidence for kin recognition and discrimination in plants and critically appraise the methods that were used. In so doing we explore different potential implications of kin recognition. The first, and the one having received most attention in the literature, is plants partly refraining from resource competition by producing smaller or less efficient resource harvesting structures when interacting with kin. Second, we move beyond the direct plant-plant resource competition and explore how kin recognition in plants may impact interactions with other organisms such as attracting pollinators, priming defense against pests and diseases and involvement of mycorrhizal symbionts, and briefly touch upon implications of these effects for population diversity and ecological community structure. Finally, we explore potential applications of kin selection crops, discussing how kin recognition and associated cooperative traits can be selected for in breeding programs.

2 Some basic concepts

2.1 Cooperation vs cheating.

An issue that is receiving increasing interest in the plant scientific literature is the conflict between individual selection and the performance of plant populations (Anten & During

2011; McNickle & Dybzinski 2013). Populations of plants with traits that are optimal in the sense of maximizing group performance are often not resistant to invasion by a more competitive plant type that invests more in resource harvesting. Evolutionary game theory predicts that populations will thus evolve towards non-optimal states. The result, i.e. a population of plants overinvesting in resource harvesting, has been denoted as a Tragedy of the Commons (TOC, Gersani et al. 2001). The classic example is plant height: short stature and associated small investment in stem tissue would favor investment in fitness enhancing characteristics (e.g. seed production), but such communities can be invaded by taller plants (Falster & Westoby 2003). A suite of other traits has since been associated with a TOC; plants may produce more leaf area, larger specific leaf area, more roots or larger flowers than what would maximize fitness of the population (see review Anten & Vermeulen 2016). The result is that vegetation stands may not be optimized in the sense of maximal performance under a given set of conditions and this may have implications for ecosystem functioning (Anten & During 2011; Farrior et al. 2015).

2.2 Kin selection when and where

As noted, however, nature is full of examples of cooperative or even altruistic behavior, that run contrary to the above-mentioned ‘selfish’ traits. Hamilton (1964) proposed that selection for altruistic behavior may occur in populations of genetically related individuals (so called kin). The basic concept is that if one helps another individual with whom one shares a certain of number of genes those genes will be passed through to the next generation through the enhanced fitness of that individual. Hamilton (1964) formalized this as a rule stating that altruistic behavior will be selected for if the product of fractional genetic relationship (r) and the fitness benefit for the recipient (B) is larger than the fitness cost incurred by the actor (C):

$$r \times B > C \quad \text{Eqn 1}$$

Hamilton's rule generally predicts that more related individuals are more likely to cooperate. But it is important to consider that, more related individuals are usually more phenotypically similar as well, compete more intensely than less related ones where differences would lead to stronger niche differentiation (Platt & Bever 2009). This raises the question as to when kin selection is likely to occur despite the existence of kin competition. It is clear from equation 1 that is the case if r and/or B are high or C is low. Equation 1 also dictates a boundary condition, being that since $r \leq 1$ ($r = 1$ if plants are genetically identical), if competition is a zero sum game, i.e., gains of one are equal to losses of the other (i.e., thus $C = B$), $r \times B$ is always smaller or equal to C and thus kin selection is unlikely to occur. In other words, if two genetically different plants ($r < 1$) compete for a fixed amount of soil resources and one plant reduces investment in resource harvesting (e.g. roots) while the other does not, and fitness costs (C i.e., acquiring less nutrients) for that plant are equal to the fitness gains (B) of the other plant, this can never have a selective benefit since $r \times B < C$. Of course, C and B need not be equal even if the resource pool is fixed. Going with the roots example, that plant produces fewer roots also reduces roots construction costs lowering C . Furthermore, in nature the target often interacts with more than one neighbor. The example however, makes it clear that kin selection critically depends on the cost and benefit of the interaction and the ecological setting. Together at least two ecological factors can be considered to favor kin selection. (i) A high r value, that is, plants tend to interact with genetically related neighbors. This is more likely to occur if dispersal rates are small and would increase e.g. with the level of self-pollination. (ii) Competition being a negative non-zero sum game entailing that altruistic behavior not only changes the competitive balance but also increases the availability of resources.

In addition, competition is not only between individual plants but also between groups of plants, especially if resources are highly mobile. Thus, even if altruistic behavior would be

selected against within a group, it can be selectively favored between groups if cooperative groups are more productive than less cooperative groups (Nowak et al. 2010). This so-called multi-level group selection may occur in the absence of kin selection, but the two are largely interchangeable, in that multi-level selection may favor kin selection (see example in Fig. 2). In both cases it is important that genetic variation in within groups is small compared to genetic variation between groups (Platt & Bever 2009).

In summary evolution of cooperative traits through kin selection is likely to occur:

- (i) When costs of altruism are low.
- (ii) When cooperative interactions result not only in less competition but also in a greater availability of resources.
- (iii) When resources are mobile making competition between groups more likely.
- (iv) In vicious plant populations in which there a clear negative relationship between genetic relatedness and distance.

2.3 An alternative hypothesis beyond inclusive fitness

Though Hamilton's rule with the key concept of inclusive fitness provides the theoretical bases for the evolution of kin selection, there are still alternative but not exclusive explanations, such as long-term benefits from repeated interactions (Dudley 2015). Game theoretical models predict that players falling into an unrepeated prisoners' dilemma scenario are prone to cheat to maximize their short-term benefits; while those in iterated prisoners' dilemma tend to cooperate for their long-term interests, as long as this accumulated benefit can exceed the costs of cooperation (Killingback et al. 2002). Therefore, reciprocal altruism can simply evolve from a group of individuals with intensively repeated interactions along their life spans, regardless of their relatedness (St-Pierre et al. 2009). In many cases, the concept of reciprocal altruism is restricted for describing the cooperation among non-kin;

however, due to the nature of dispersal, siblings from the same mother or individuals from the same family are more likely to have overlapping living spaces, creating high chances of repeated interactions. Thus, reciprocal altruism is still more likely to occur among relatives, potentially leading to the evolution of kin selection in plant species, especially those with viscous population structures.

3 Kin recognition and resource competition

3.1 Evidence for kin recognition at root level

Research in the last two decades is starting to show that changes in root traits of a given plant may occur in response to not just the presence but also the identity of neighbor plants. Early works in this regard addressed whether plant roots can distinguish whether roots, with which they come in contact, are from self (the same plant) or non-self (a genetically different plant, e.g. Gersani et al. 2001; or even a genetically identical but physiologically disconnected plant, e.g. Chen, Vermeulen et al. 2015). They showed that non-self-interacting plants demonstrated a tragedy of commons in terms of more root allocation for soil resource competition at the expense of less reproductive allocation than purely self-interacting ones. While their methodology was criticized (e.g. Hess & de Kroon 2006; Chen, During et al 2015 and see Chen et al. 2012 for review), it clearly spurred a wave of interest in identity recognition at root level.

The first study to document root-mediated kin recognition was from Dudley & File (2007). They grew *C. edentulata* plants either solitarily or paired with a half-sibs or strangers in pots, and found that stranger interacting plants produces more roots than both solitary plants and half-sib interacting ones. While there was no effect on seed production, it was viewed as evidence for kin recognition in plants. Similar results were since obtained in species ranging from gymnosperms (e.g. *Cycas edentate*, Marler 2013) to angiosperms

(including both wild species, e.g. *Ipomoea hederacea*, Biernaskie, 2011; *Deschampsia caespitosa*, Semchenko et al. 2014; and crops, e.g. *Triticum aestivum*, Zhu & Zhang 2013; *Glycine max*, Murphy et al. 2017) with plants in non-kin interaction producing (i) more roots or (ii) roots with more competitive morphological traits, e.g. more lateral root number (*Arabidopsis thaliana*, Palmer et al. 2016), greater root branch density and specific root length (Semchenko et al. 2014) as well as higher degree of root confrontation (*Oryza sativa*, Yang et al. 2018, but see Fang et al. 2013), or (iii) roots with higher physiological activities, e.g. higher nutrient uptake rate (Zhang et al. 2016) and water uptake rate (Takigahira & Yamawo 2019). However, other studies also observed no differences between kin and non-kin interaction (*Lupinus angustifolius*, Milla et al. 2011), or kin interacting plants even producing more roots (*Distichlis spicata*, Mercer & Eppley 2014).

Interestingly, in addition to the aforementioned root responses, belowground kin recognition also has consequences on the aboveground competitive performance of plants. For example, when understory species *Impatiens pallida* plants rooted with kin neighbors, they tended to reduce mutual shading by producing taller stems with more branches but less leaves (Murphy & Dudley 2009). Similarly, a cease of competition for light resources was observed in *Fagus crenata* seedlings by producing leaves with less chlorophyll content (Takigahira and Yamawo 2019), and in *Lychnis flos-cuculi* by producing leaves with lower specific leaf area (Lepik et al. 2012) when these plants have root interactions with a kin as compared to a stranger.

3.2 Mechanisms of kin recognition between roots

How do plant roots can detect the level relatedness of other plants? Work so far clearly suggests the involvement of root produced exudates. To date, more than 200,000 plant secondary metabolites are identified, a great portion of them are secreted as root exudates into

the rhizosphere (Kessler & Kalske 2018). Plant individuals typically secrete a wide variety of them, and the composition of exudates differs between species and between genotypes within a species (Mommers et al. 2016). First evidence for the role of exudates in kin recognition, came from Biedrzycki (2010) showing stronger lateral root growth of *A. thaliana* seedlings when exposed to exudates from stranger roots than from sibling's, this difference disappearing when a root secretion inhibitor was added to the exudates solution. ABC transporter genes, which are involved in secretion of secondary metabolite compounds, appear to participate in the process (Biedrzycki et al. 2011). More recently, Yang et al. (2018) also found that rice seedlings growing in solutions with non-kin exudates produced more roots than those growing in solutions with kin exudates. Semchenko et al. (2014) took a somewhat different approach, collecting leachates from pots with different *D. caespitosa* plants and feeding these leachates to kin or stranger plants. Plants exposed to leachates from strangers produced more branched roots with higher specific root length than those exposed to kin leachates. They found plants growing in non-kin solutions to produce more roots. All these studies clearly indicate that exudates play a role in kin recognition at root level.

Clearly, the role of exudates in kin recognition seems to be evident in a general sense, but which compounds, or combinations of compounds are involved, and do they operate in a dose dependent way? To our knowledge so far only one study (Yang et al. 2018) has specifically considered this question. They found secretion of the compound allantoin, a N rich compound involved in various plant metabolic processes, to be higher when rice plants interacted with plants of a different cultivar than with the same. They also showed that an exogenous application of allantoin, caused changes in auxin synthesis and gene expression in the focal rice plants. These findings clearly demonstrated that allantoin production is mediated by the level of kin in the interaction and that the level of allantoin production is linked to the kin responses that were observed. Meanwhile, some recent neighbor detection

studies also showed that lactone (e.g. (-)-loliolide) and phytohormones (e.g. jasmonic acid and salicylic acid) in the root exudates of various weed species were the key chemicals that are sensed by allelopathic wheat and rice, and can elicit them to produce more phytotoxins (Kong et al. 2018; Li, Xia et al. 2016; Li, Zhao et al. 2020). However, to what extent these chemicals are also involved in the kin recognition of wheat and rice is still unknown. Together, evidence is clearly mounting that plant roots are capable of kin recognition and that this may involve a wide variety of root as well as aboveground traits. Root exudates likely play a role though more work is needed to identify (combinations of) compounds involved.

3.3 Evidence and the associated mechanisms for kin recognition aboveground

Compared to belowground interactions, aboveground interactions between plants have been extensively studied for decades, ranging from signal perception to responses of gene expressions, physiological processes and to ecological impacts (Pierik et al. 2013). The best studied example is shade avoidance syndrome (Pierik and de Wit 2014), in which plants detect the presence of a neighbor via the changes in the spectrum of light reflected from neighbors and typically respond with stem elongation, reduced branches and changes in leaf angle. But can plants determine kinship based on such light signals?

To answer this question, Crepy & Casal (2015) grew *A. thaliana* either in con-accession groups or hetero-accession groups, and found that plants actively reoriented their leaves to avoid mutual shading with neighbors only in con-accession group, and that this was associated with higher seed production than in hetero-accession groups. These results were interpreted as evidence of photoreceptor-mediated aboveground kin recognition. But it could also have involved phenotype matching, light signaling phenotypic similarity, which is correlated with genetic relatedness (Till-Bottraud & Villemereuil 2015).

In addition to light signals, plant volatiles may play a role as well. Ninkovic (2003) grew barley (*Hordeum vulgare*) plants of two varieties, Kara and Alva, and exposed plants to volatiles of the same or the other variety. Kara plants allocated more biomass to roots and less to shoots when exposed to volatiles from Alva than when exposed to their own. In addition to the constitutively emitted volatiles in the barley example, biotic attacks also can stimulate plants to temporally emit special volatiles as “alarming calls” that can be sensed by the un-attacked parts of the same plant or its neighbors to prime their defense before being attacked (Markovic et al. 2018). Interestingly, for some species the level of defense priming seems to be stronger in kin recipients than in non-kin recipients (Karban et al. 2013; Hussain et al. 2019 and see further Section 4.3). In general, although plant-plant aboveground communications have been well studied for decades, researches of kin recognition aboveground, compared to belowground, so far are still limited, and sometimes remain controversial.

4 Kin recognition beyond resource competition

4.1 Pollination: cooperating to enlarge the resource pool

The examples in the previous sections dealt only with the level of competition for abiotic resources (water or nutrients), cooperation arising from putting a smaller demand on the common resource pool. In the current case, pollination, we expand this to the idea that cooperation may help expand the resource pool.

Plants do not only compete for light, water and nutrients but, at least in some cases, also for services from other organisms, e.g., pollinators and seed dispersers. Generally, pollinators will be attracted to larger flowers, but investment in large petals also costs energy. One could thus imagine there to be an optimal flower display size where the difference between population-level benefits (attracted pollinators) and costs (the investment in petals going at

the expense of other functions) are maximized. Based on concepts of individual selection one may argue that such populations might be prone to invasion by cheaters with larger than optimal flower displays thus attracting a larger share of extant pollinator population, a TOC (McNickle & Dybzinski 2013; Anten & Vermeulen 2016).

The question is whether and how kin interaction could influence investment in flower display. In a recent study Torices et al. (2018) addressed this question by growing individuals of the insect-pollinated herb *Moricandia moricandioides* groups of either with half-sibs or strangers, and measured flower display size (flower number and petal biomass). They found that kin groups produced larger flower displays than stranger groups. At first glance, this result seems surprising, plants investing more in competition for pollinators in kin than in non-kin groups (Fig. 2a,b). However, flower size might, at least under some conditions, be prone to group selection. In foraging, pollinators might initially be looking for patches of flowering plants, and only in a later stage select at the individual plant or flower level, or simply visit every flower in a patch. Under natural conditions, *M. moricandioides* is known to grow in patches (Torices et al. 2018). Then, increasing flower size also acts to increase the pool of pollinator for a group of plants, at the expense of other groups, and thus larger flower size could be interpreted as cooperative traits within the group but competitive between groups (Fig 2c). Torices et al. also found that the kin effect on flower display was greater at high than at low plant density. Plants at high density compete more for light, water and nutrient and competition for these resources tends to be within rather than between groups. Evidently more work on kin interaction and flower display is needed to explore the generality of Torices et al.'s findings. But the results do suggest that plants in kin interaction may shift allocation from structures that harvest local resources to ones that harvest more global ones and for which competition with other groups is more likely.

4.2 Mycorrhizal networks: investment in a common resource harvesting network

This example builds on the previous one, cooperation helping to enlarge the resource pool, but rather than doing this through a plant's own structures, it involves investment in a common symbiotic network. The second expansion involves the idea that kin recognition may involve not only cues produced by plants but also other organisms that convey this cue.

Plants form symbiotic associations with mycorrhizal fungi whereby the mycorrhiza provide water, nutrients and/or pathogenic defense to the plants and the plants provide carbon (Smith and Read 2008). One emerging property in this symbiosis is the formation of common mycorrhizal networks (CMN) that connect different plants that transfer N, water and even carbon between plants. Such CMNs thus entail a common good: carbon investment of each plant into the CMN allows the mycorrhiza to grow larger and exploit larger volumes of soil acquiring more water and nutrients. If these resources are limiting, this investment in turn provides a common benefit (Rankin et al. 2007). In theory this system is open to cheating, and associated tragedy of the commons (File et al. 2013; Wyatt et al. 2014). If a plant unilaterally provides less carbon, it will reduce costs while the resulting reduction in goods provided by the CMN are spread over the connected population, thus giving the cheater a relative benefit. Such selection for cheating can lead to collapse of the CMN. One way that stable symbiosis in CMNs can be enforced is through sanctioning, if fungi detect differences in carbon supply by plants and make nutrient provisioning dependent on it (Wyatt et al. 2014). Kin selection however would be an alternative, there being added selective advantage to invest in a network shared with kin. Investment in CMNs is cooperative behavior that increases the total pool of resources, making the interaction a non-zero sum game whereby costs incurred by investing in combined network are smaller than the accumulated benefit.

To explore whether kin recognition may be involved in the functioning of CMN's, File et al. (2013) conducted experiments where *Ambrosia* plants were grown either with half-sibs

or offspring from different mothers with or without mycorrhizal fungi. The size of the mycorrhizal networks, expressed as soil hyphal length and levels of root colonization, were greater in half-sib groups than in groups of strangers. Half-sib groups also benefited more from the presence of a CMN e.g. in terms of P uptake and suppression of pathogens. These intriguing results of kin recognition and possible kin selective effects operating via mycorrhiza raise questions as to the mechanism of kin recognition. File et al. (2013) proposed that it could involve direct kin recognition, whereby plants recognize the presence of kin through exudates released by those plants into the soil, or through exudates actually being transported through the mycorrhizal hypha, which induce them to invest resources into the CMN. The mechanism however could also be less direct, kin recognition at root level inducing changes in root architecture or anatomy making mycorrhizal colonization easier. Or, if kin interacting plants are already doing better in terms of assimilation, they will have more resources to invest in CMNs (Fig. 3).

To further explore whether kin related signals might be directly communicated via mycorrhizal hypha, Pickles et al. (2017) grew full-sib- and stranger seedling pairs of Douglas fir in pots and fed one plant of the pair with ^{13}C labelled CO_2 and measured how much carbon was allocated into the ecto-mycorrhizal fungi (EMF) biomass or transferred to the paired seedling. They found that levels of ^{13}C in the mycorrhizal biomass as well as in recipient plants were higher in kin pairs than in stranger pairs. They concluded that these could indicate the presence of signaling compounds being transferred. Research on ecto-mycorrhizal host-symbiont interface reveals that EMF produce signaling compounds, which are translocated into plant cell nuclei to alter the host transcriptome, e.g. to promote ectomycorrhizal formation and reduce jasmonic acid production (Plett et al., 2014). The results of Pickles et al (2017) thus suggest that the greater carbon exchange between kin could have come due to

increased inter-root activity as a result of increased transfer of signaling compounds and/or micronutrients (Babikova et al., 2013; Plett et al., 2014).

Even though much more research is needed, these results together provide evidence that kin recognition between plants may involve another organism that acts both as a messenger for recognition and as a reward in kin selection. In the case of CMN's the cooperative strategy (i.e., investing resources in the CMN) increases the carrying capacity for the plant population through enhancement of resource availability. As noted in Section 2, this in turn can be an important condition to favor kin selection. But questions remain. Are the carbon compounds transported via the CMNs really involved in kin recognition or is it that recognition first occurs at the root level e.g. via exudates subsequently inducing plants invest more in CMN's? How widely does this type of interaction occur in plants and how important is it in sustaining stable CMNs under natural condition?

4.3 Defense: volatile communication about danger

Above examples all dealt with resource competition; kin selected cooperative traits that reduce competition or help attract more resources to the group. Mutual benefits among plants may however also arise in other ways, for instance, through signals that warn about impending danger. An example of the latter involves plant-plant communication in relation to insect herbivory.

When plants are attacked by insect herbivores they produce so-called herbivore-induced plant volatiles (HIPVs), which tend to attract natural enemies of these herbivores, the response thus having been dubbed a cry for help (Bruin & Dicke 2001). However, these volatiles can also be detected by neighbor plants (i.e., receiver plants), and it has been shown these receivers may increase their levels of defense and suffer less herbivory damage than plants that are not exposed (see review Karban et al. 2014).

Part of the debate about this volatile communication has centered around the question whether it involves a simple eaves-dropping strategy that is only beneficial to the receiver or whether there is some adaptiveness to the emitter as well (Karban et al. 2014). One possibility is that volatile communication may originally have evolved to help coordinate self-regulated systemic responses, it being more effective than signaling through the plant's vascular system. Many plants also propagate vegetatively and may form patches of ramets all pertaining to the same genetic individual. It was shown that plants respond more strongly to self-produced than to non-self-produced HIPVs (Karban & Shinjori 2009). This raises the question of whether plants are also capable of discriminating HIPVs produced by kin from those of strangers.

Karban et al (2013) conducted several experiments with Sagebrush *Artemesia tridentata*. In one they placed potted emitter plants in patches of soil-grown receivers that varied in the extent to which they were related to the emitter. In another more manipulative experiment they found a consistent pattern whereby plants exposed to HIPVs from kin suffered less herbivore damage than those exposed to non-kin. The experimental set up with emitters growing in separate pots or direct HIPV application eliminated the possibility of the communication being via root produced exudates, and thus indeed implicated the composition of the HIPVs being the signal. Hussain et al. (2019) found volatile signals from beetles-attacked lodgepole pine (*Pinus contorta*) trees can only triggers the defense priming, in terms of higher concentrations of monoterpene compounds, of genetically related neighbors and argued that this served to prevent eavesdropping from non-kin neighbors. It is known that plants usually produce a wide variety of HIPVs (Bruin & Dicke 2001) but it is still unclear what aspects of the composition elicit responses in plants (Douma et al. 2019). Karban et al. (2013) did find a correlation between similarity in HIPV composition and genetic relatedness, which suggests that plants in kin interaction, plants responded more strongly simply as the HIPVs more strongly resembled their own, but more work on this is needed.

4.4 Kin recognition in a broader ecological context

The effects of kin recognition on the performance of plant individuals also have consequences on the dynamics and structures of plant populations and communities. For example, the preferential transfer of photosynthates to siblings than to strangers via CMN in Douglas-fir (Pickles et al. 2017) can provide siblings with competitive advantages against unrelated neighbors. Recent evidence suggests that kin recognition can even directly facilitate interspecific competition. In competition with *Trifolium repens*, sibling pairs of *Plantago asiatica* directed more leaves towards *T. repens* than stranger pairs. As a result *T. repens* grew smaller with sibling pairs of *P. asiatica* (Yamawo & Mukai 2020), though this cooperative leaf orientation towards strangers might also be a by-product of the avoidance of mutual shading between siblings. In addition to the role of enhancing resistance of plant groups against intruders, there is also evidence that kin recognition may facilitate the invasion success of exotic species as well. Individuals of the South American alligator weed (*Alternanthera philoxeroides*) introduced to North America grew larger in intra-genotypic interaction than in inter-genotypic interaction; but the trend was opposite for native genotypes in South America (Zhang et al. 2019). Obviously, these kinship dependent facilitative effects including support and cooperation potentially can affect the distribution of genetic diversity in plant populations by reducing local genetic heterogeneity (Tedersoo et al. 2020).

Kinship-dependent facilitation is probably also habitat dependent. For instance, facilitation, in terms of higher survival rates in intra-genotypic than in inter-genotypic competition, in *Medicago rigidula* seems to only occur when plants were grown in soils collected underneath allelopathic thyme shrubs (Ehlers et al. 2016). Conversely, such a facilitation in the con-generic species *M. minima* only occurred when plants were grown in non-thyme soil. There is further evidence that the extent of kin discrimination is correlated

with the nutrient availability in the habitats. For example, reduced root activity and nutrient uptake in sibling-pairs compared to non-sibling ones in sorghum occurred under low but not under nutrient availability (Li et al. 2018). Similarly, intra-cultivar interacting peas produced more seeds than in inter-cultivar interacting ones only at low nutrient availability (Pezzola et al. 2020). Palmer et al. (2016) further demonstrated that N and P are the key elements determine nutrient availability effects. They grew *A. thaliana* seedlings either solitarily or paired with a sibling, a relative (from a different mother in the same accession), or a stranger (from a different accession) in agar with Murashigie-Skoog nutrient media. They found that stranger-paired plants had more lateral roots than other plants only when nutrient strength was lower than 0.75 strength. Relative- and sibling-paired plants produced more lateral roots than solitary plants only when the solution was further diluted. They could mimic these results by only removing N and P from the full strength solution but not other elements, showing that kin recognition depends not only on the amounts of nutrient but also their composition.

Clearly kin recognition may have implications for the structure, diversity and functioning of communities likely extending to the ecosystem level that we are only beginning to unravel. There is thus a clear need for systematic ecological field experiments across different systems and species, where genetic relatedness in populations is manipulated or at least known and a broad scale of community interactions are explored. Moreover, kin discrimination seems to be more important in regulating plant growth and community structure under stressful, especially infertile, conditions. This could imply that species originating from stressful habitats might be more likely to possess the ability of kin recognition, but this needs to be tested.

5 Cautionary in current research

5.1 Methodological concerns about root studies

A methodological concern with pot-based studies of root-mediated kin recognition is that in most cases roots of individual plants could not be separated and root mass needed to be measured at the pair (or group) level. This implies that the larger individual(s) contribute more to the results than smaller ones. Size inequality is likely to be larger in non-kin than in kin interactions. Biomass allocation is at least to some extent size dependent, with larger plants typically investing proportionally more in support (stems and coarse roots) and less other parts (leaves, fine roots and potentially reproduction). The larger reproductive effort or smaller proportional root investment observed in kin-interacting plants could thus be the result of their sizes being more similar, rather than the product of kin recognition (Klemens 2008). In addition, Jensen's inequality effect suggests that, when plant size and fitness follow a curvilinear saturating relationship and competition between plants with greater differences in relatedness yields larger size asymmetry, plants in non-kin interactions will naturally generate lower group fitness than those in kin interactions independently of kin recognition (Ehlers & Bilde 2019; Simonsen et al. 2014). A pair-wise family design (Bhatt et al 2011), whereby all combinations of families (either plants of the same [kin] or different [non-kin] family) are tested and differences in competitiveness are corrected for, could be a solution for the problem size inequality but does solve the bias from Jensen's effect.

Some studies (Fang et al. 2013; Yang et al. 2018) used pots filled with transparent substrate e.g. agar or Yoshida rice solutions, allowing them to also study kin effects on root architecture. A problem however is that such solutions are very different from soil, i.e., typically having lower oxygen levels and higher mechanical resistance. It is entirely unknown whether such differences may somehow interact with the kin vs non-kin contrast.

5.2 Relation with diversity and complementarity

Most of the work on kin recognition discussed in this paper concludes that genetically related plants exhibit more cooperative traits leading to higher group performance. But such plants are also phenotypically more similar, and classic ecological theory dictates that these plants exhibit less niche differentiation and complementarity, leading to stronger competition (Chase & Leibold 2003). Thus, kin effects and complementarity effects are to some extent confounded and this may have complications for interpretation of experimental results. For instance, higher root mass productions observed in non-kin interaction as compared to kin interaction, can also be a divergence in root vertical distributions or in mineral nutrient demands contributing to niche differentiation. The outcome of kin interaction, as compared to non-kin interaction, in most cases is likely determined by both kin recognition and niche effects (Ehlers & Bilde 2019). Depending on the relative size of two opposite effects, the outcome of kin interaction can be positive, neutral even negative as compared to non-kin interaction. As one can partly mask the other it could lead to false negatives, e.g. wrongly concluding that kin recognition does not exist.

5.3 A possible solution to bypass confounding factors

Most of the concerns mentioned can be attributed to the fact that it is impossible to exhaust all biotic and abiotic environmental determinants and separate kin recognition effect from them in a real plant-plant interaction. While mechanism-driven approaches may provide some hints that help us to some extent bypass some confounding factors listed above. For instance, in the studies of below- and aboveground kin recognition, we can respectively expose focal plants to the key signals, i.e. root exudates and volatiles, manually collected from kin or non-kin neighbors rather than expose focal plants to neighbors *per se* (e.g. Biedrzycki et al. 2010; Mercer and Eppley 2014; Semchenko et al. 2014). This method has already been successfully applied, as already well described in Sections 3.2 and 3.3. But the collection of root exudates,

in terms of exudate extracts, leachates or solutions, used may differ not only in the composition of exudates supposedly involved in kin recognition but also in e.g. composition of nutrients, and these effects may differ between genotypes. Thus, we suggest that an adjustment of nutrient contents, at least the key elements N and P (Palmer et al. 2016), should be made for the collection of exudate solutions before treating focal plants with these solutions. Moreover, using sufficient replication at the genotypic level however would probably overcome any bias that may arise from this.

5.4 More field evidence needed

All studies mentioned so far were essentially greenhouse pot studies, and translation from such studies to the field is notoriously difficult especially when the work involves roots. Yang et al. (2018) conducted a double experiment with rice whereby different combinations of kin (same variety) and non-kin (different varieties) were grown both in pots in the greenhouse and under natural condition in the field. Results were very consistent; both in conditions plants interacting with kin produced fewer roots and had greater seed production than those growing with non-kin. While more work needs to be done, it does suggest that kin recognition operates under field conditions and can influence plant community performance. This has important implications for both field ecology and crop science (the latter discussed in the next section).

6 Kin selection and crops

Farmers generally aim to increase crop yields (or some other performance measure such as resource-use efficiency) at the crop stand level. This entails that crops would need to have traits that enhance group rather than individual performance. Donald (1968) first recognized this stressing that crop plants should be selected for cooperative rather competitive traits, and this point was echoed in recent reviews (e.g. Weiner 2010; Denison 2011; Anten &

Vermeulen 2016). There appears to be evidence that modern crop selection may have to some extent unwittingly (i.e., without formal knowledge of how natural selection operates) done this. The most notable example is the shortening of several of our major cereal crops (e.g. wheat and rice) during the green revolution. But there may be ample scope for further crop improvement especially in traits other than stature such as the size and architecture of root systems, allocation to defense and symbiotic relationships with soil biota that are less visible (see review Anten & Vermeulen 2016). As discussed so far, kin selection can lead to more cooperative traits in plants and greater group performance. In addition, compared to natural plant communities crop stands are often composed of strongly related plants. The question thus arises is whether selection for kin recognition and discrimination can create more altruistic crops.

This raises two questions: (i) has kin discrimination been selected for unwittingly and (ii) can it be actively facilitated in breeding programs.

Regarding the first question, kin discrimination has been documented in several crop species, e.g. in soybean (Murphy et al. 2017), barley (Ninkovic 2003), sorghum (Zhang et al. 2016), wheat (Zhu and Zhang 2013, but see Fréville et al. 2019), rice (Yang et al. 2018) and sunroot (*Helianthus tuberosus*, Fukano et al. 2019), with kin-interacting plants tending to exhibit more cooperative root systems. But this does not exclude the possibility that this kin discrimination was inherited from the wild ancestors of these species. Evidence that kin discrimination may have been unwittingly selected for requires researchers to find that newer higher yielding varieties exhibit greater kin discrimination than older lower-yielding ones. Some evidence for this comes from a study on wheat (Zhu and Zhang 2013). These authors grew an old variety (Monkhead common in China between 1940) and a newer one (92-46 used in the 2000s) in a classic De Wit replacement series. They found that root systems of 92-46 plants were progressively smaller when interacting more with other 92-46 plants and larger

when interacting with Monkhead. Monkhead, on the other hand, did not exhibit this plasticity. This suggested the more modern variety 92-46 to be more capable of kin discrimination than the older one. This, however, is the only study that we know of that has compared kin discrimination in varieties of different release dates. Furthermore, the study was limited as it involved only two cultivars; the trend could have been purely coincidental. Much more replication at the genotypic level is needed to determine whether indeed there is a correlation between kin discrimination and release date.

The question of whether kin selection can be actively engaged in crop breeding needs to consider the broader objectives of- and constraints on crop breeding programs. Ideally, one maximizes the chance of finding the variation that can be used to select for improved plant performance over a range of conditions by using as many genotypes in as many conditions possible in the realistic and high plant densities used by farmers (Anten & Vermeulen 2016). The most traditional way in this regard is mass selection, selecting the best performing individuals from populations with a very large number of genotypes (Fig. 4a). This type of selection was important in crop domestication and still plays a role in modern crop selection. The problem with this type of selection is that it is individual-based and thus would favor plants with competitive rather than cooperative traits. The alternative is using some form of group selection that would tend to favor selection for cooperative traits (Fig. 4b). This methodology could help select for kin recognition in crops if groups are restricted to kin (Murphy et al. 2017). The drawback of that approach is that it requires more space and time and thus limits the number of genotypes that can be tested in a given space or time.

Typical phenotypic selection programs tend to be a hybrid of individual and group selection. After a crossing, mass selection occurs in the first couple of generations and then gradually favorable genotypes are planted in lines to see how well they perform in monocrop. As shown by Murphy et al. (2017) such programs could be modified by conducting group

selection earlier on (e.g. already in the F2 generation) and more readily planting offspring of individual plants in the same group (Fig. 4b). As noted, however, such an approach would be more space and time demanding and whether it would be feasible would depend on the crop performance improvement that can potentially be achieved through kin discrimination (Fig. 5). As such it is important to note that while kin discrimination has been documented in a wide variety of plant species and traits, the magnitude of the performance benefit has not been well documented. In this regard it is important to consider the earlier mentioned findings that kin recognition seems to be mostly apparent under nutrient limited conditions. This could entail that kin recognition in crops might have the most promising results in low input farming.

A final point of consideration is that the use of kin recognition in crops inevitably means low diversity crops. As noted, in so doing one foregoes the potential benefits of diversity (e.g. niche differentiation in resource acquisition or variation in disease resistance). Interestingly much work on making agriculture more sustainable through improvement of ecological relationship in crop systems has focused on enhancing diversity, while the role of kin recognition has not been considered. Clearly much more research is needed to indicate when, where and by how much kin recognition can contribute to increasing yields or other crop functions. This could include modelling studies that explore the extent to which selection for cooperative traits could contribute to yield increases.

7 Conclusions and outlook

While the idea of kin selection and its underlying components, kin recognition and discrimination, have been around since the 1960s (Hamilton 1964) and have been described in many life forms including very primitive ones (Platt and Bever 2009), it only recently emerged in relation to plants (Dudley & File 2007). But since the pioneering work of Dudley

& File (2007), kin discrimination has been documented for a variety of traits and types of plants. Many cases have been documented of plants exhibiting more cooperative behaviors towards kin than towards strangers. These behaviors can take the form of reducing competition for a common resource pool, enhancing the size of that common pool or conveying information about impending threats. While concerns still remain about some of the methods used, which need to be addressed, it is probably safe to say that kin recognition in plants exists. But research on the mechanisms of kin recognition has so far been limited. Overall, with one exception, the implication of variation in alantoin production in rice (Yang et al. 2018), knowledge has gone no further than the idea that in general root exudates and plant volatiles play a role. More biochemical and molecular research is needed to identify the chemical cues that associate with or signify genetic relatedness and how and where this is sensed in plants. Other possible mechanisms (Fig. 1), such as physical touch between leaves (de Wit et al. 2012), acoustic emission (Mishra et al. 2016) and electrical signaling in soils (Volkov et al. 2019) that observed in the studies of neighbor detection and inter-plant communication also deserve investigation. Another issue is that much of the work so far has been conducted with rather limited sets of genetic material and often being limited to siblings versus stranger without quantifying genetic relatedness (but see e.g. Karban et al. 2013). Expanding on this will not only help overcome some of the experimental biases that have been associated with research on kin discrimination (e.g. Bhatt et al. 2010; Semchenko et al. 2014; Ehlers & Bilde 2019) but would also give a much better indication of the level at which plants can detect genetic relatedness. Kin recognition may also have far-reaching implications for the structure and composition at the community level as it can modify multi-trophic interactions and as it potentially can select for population with lower diversity. Much more field experiments are needed to explore these effects. Finally, the existence of kin discrimination holds potential for breeding more cooperative crops that could form more

highly productive resource efficient and resistant crop stand. But designing phenotyping programs that adequately facilitate kin selection is costly and it is thus important to assess the magnitude of potential positive effects that may arise from kin discrimination.

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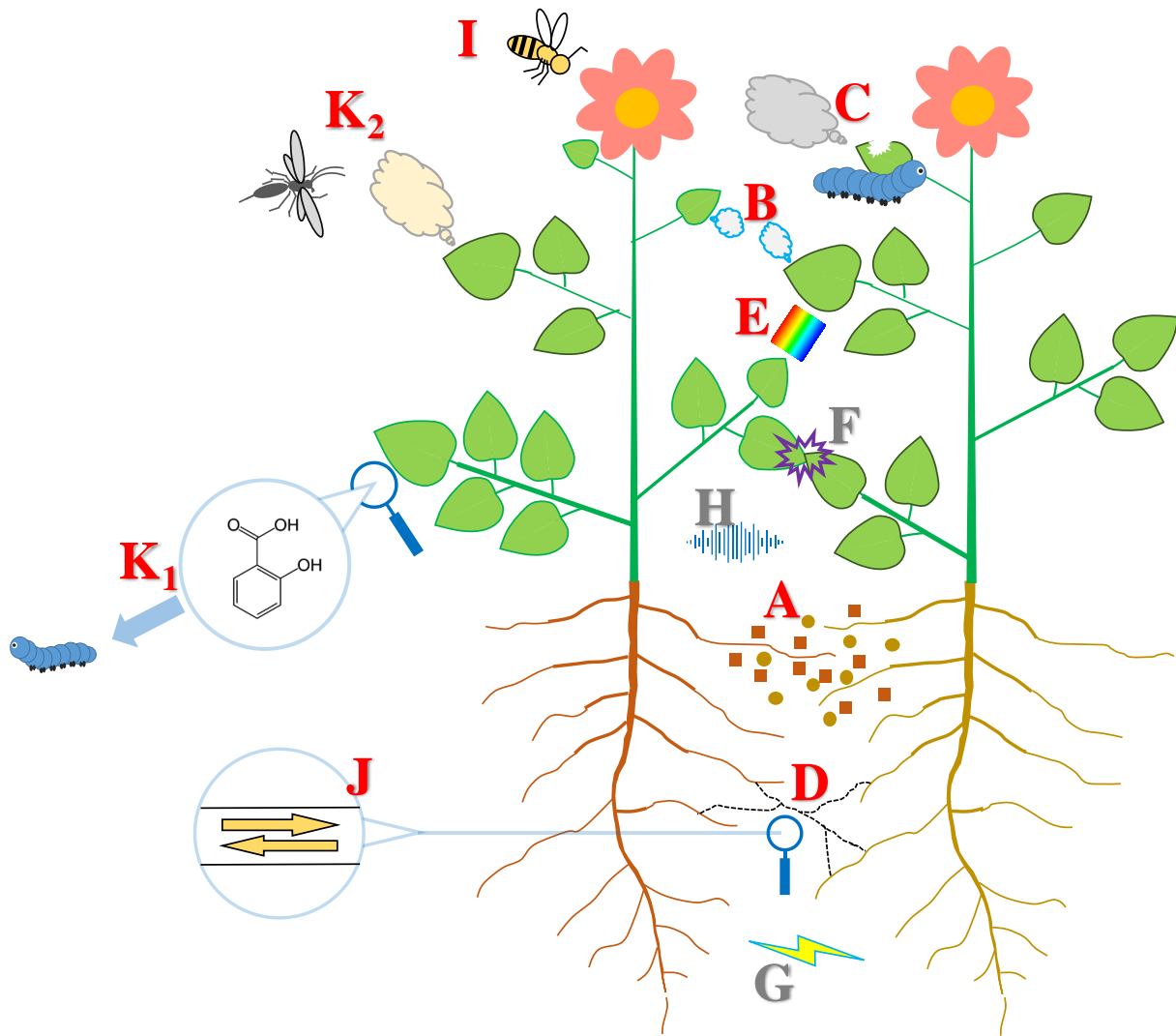


Figure 1 Summary of the physiological mechanisms for kin recognition and the associated kin discrimination (beyond resource competition) in plants. Signals and pathways that are proven to mediate kin recognition include (A) root exudates and volatiles (B) emitted constitutively or (C) induced by biotic attacks, and probably also include (D) common mycorrhizal network and (E) light spectrum. Other potential signals and pathways that mediate plant neighbor detection and inter-plant communication but have not been tested for kin recognition include (F) leaf physical contact, (G) electrical signal transmission, and (H) sound emission from xylem during drought stress. Kin discrimination behaviors that are beyond well-discussed resource competition in plants include investments in (I) pollinator attraction, (J) inter-plant transfer of photosynthates and mineral nutrients via common mycorrhizal network, and defense priming, in terms of (K₁) phytotoxin production and (K₂) natural enemy attraction, induced by neighbours' alarming volatiles.

Neighbor recognition in plants

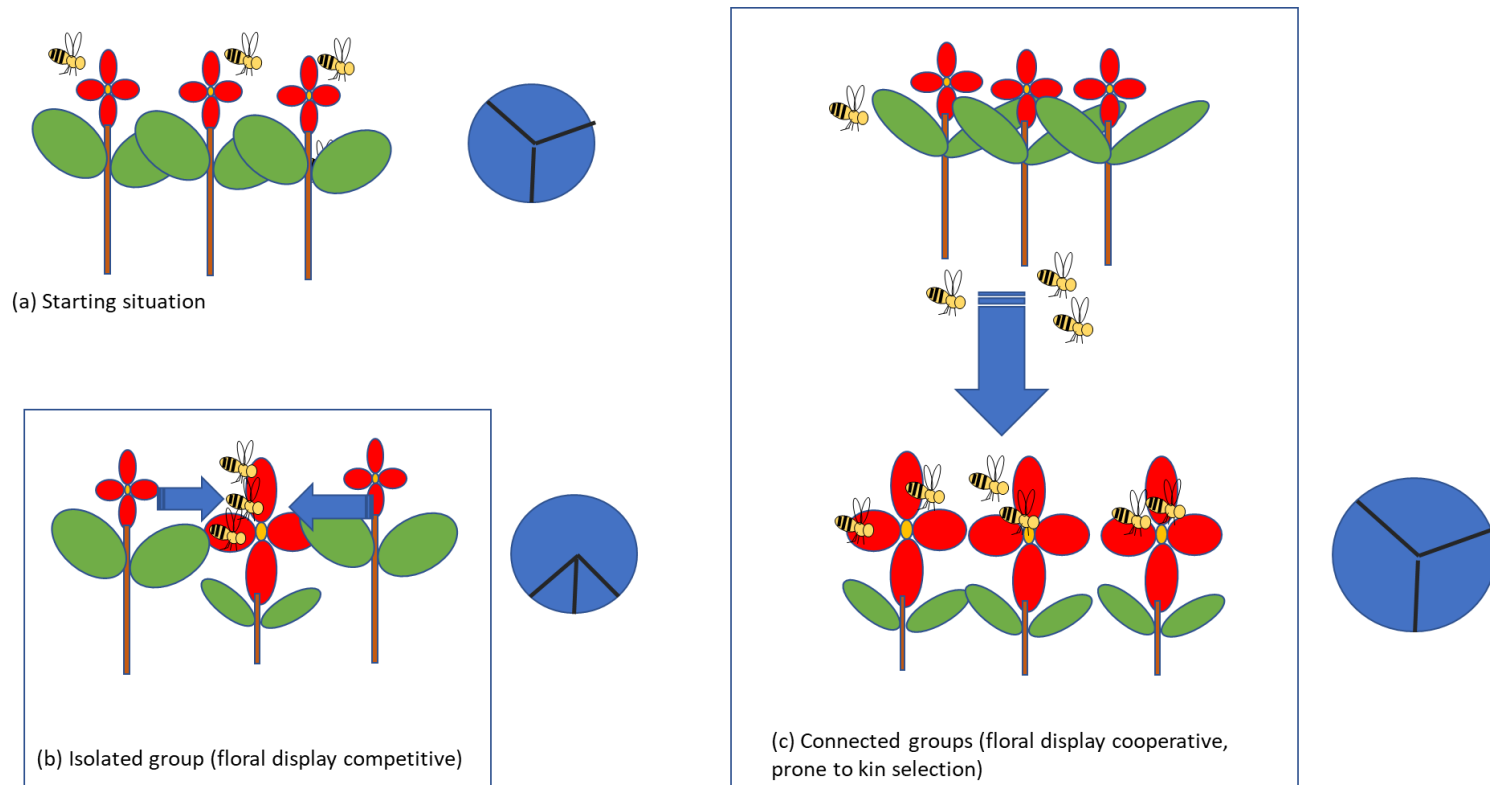


Figure 2 A depiction showing that interpreting floral display as a purely competitive or partly cooperative trait (and thus prone to kin selection) depends on meta-population structure (inspired by Torices et al. 2018). Plants invest resources in floral display allowing them to compete for pollinators, but this investment goes at the expense of other functions (e.g. leaves and roots to acquire physical resources including light and soil water and nutrients). (a) A starting situation with plants all having the same relatively small floral display and pollinators equally divided over them (equally divided blue pie). (b) Individual selection: Within an isolated group of plants, a larger display by one individual entails that it attracts a larger share of the same pollinator population as in (a), i.e. same size blue pie unequal division at the cost of producing a smaller

vegetative shoot. If pollination is limiting fitness more than physical resource acquisition, this individual can invade the population arises with larger flowers, but the same numbers of pollinators (this end situation not shown). Thus, floral investment could be viewed as a cheating strategy leading to a tragedy of the commons (Anten & Vermeulen 2016). (c) Here a group of plants is connected with other groups (pollinators forage between these groups). Investment in floral display by all plants in one group attracts pollinators away from other groups thus increasing the resource pool (larger pie, equal division), larger floral display leads to more pollination. At the group level, floral display can be and can be viewed as a cooperative strategy (attraction of more pollinators helps the whole group) and can be prone to kin selection.

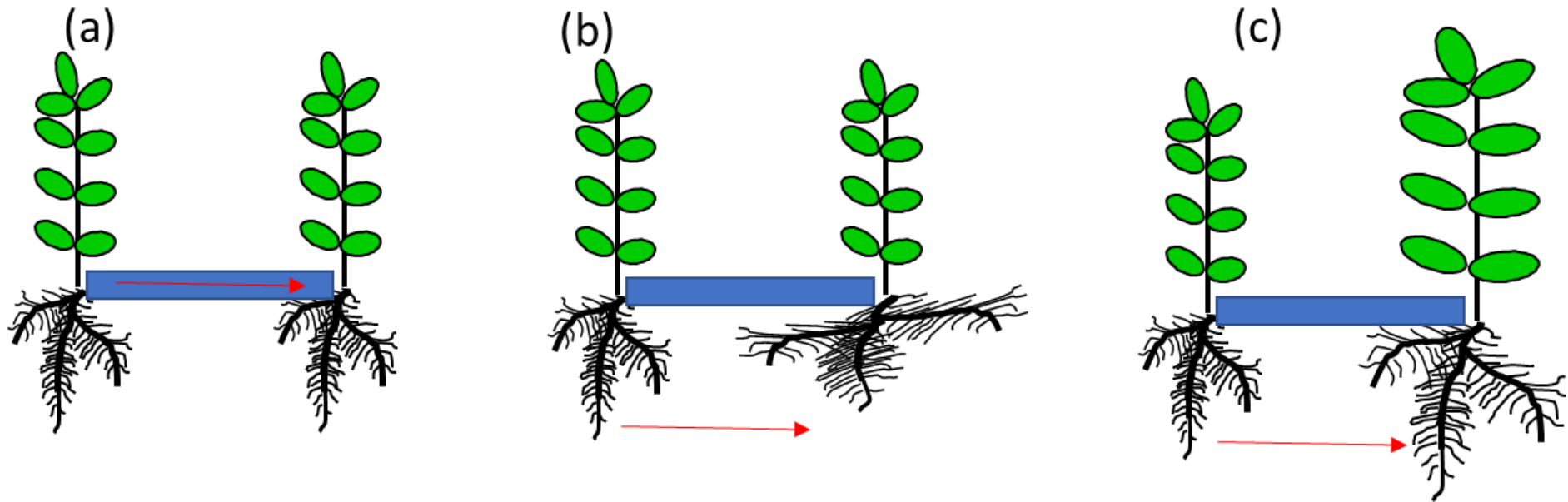


Figure 3 Alternative pathways through which kin recognition can result increased investment in a common mycorrhizal network (CMN). (a) The kin related signal(s) (red arrow) go directly through the mycorrhizal hypha (blue bar); (b) kin related signals are communicated by roots inducing changes in root architecture that it infection by mycorrhiza easier and (c) kin related signals are communicated by roots inducing greater performance and hence more resources to allocate to the CMN.

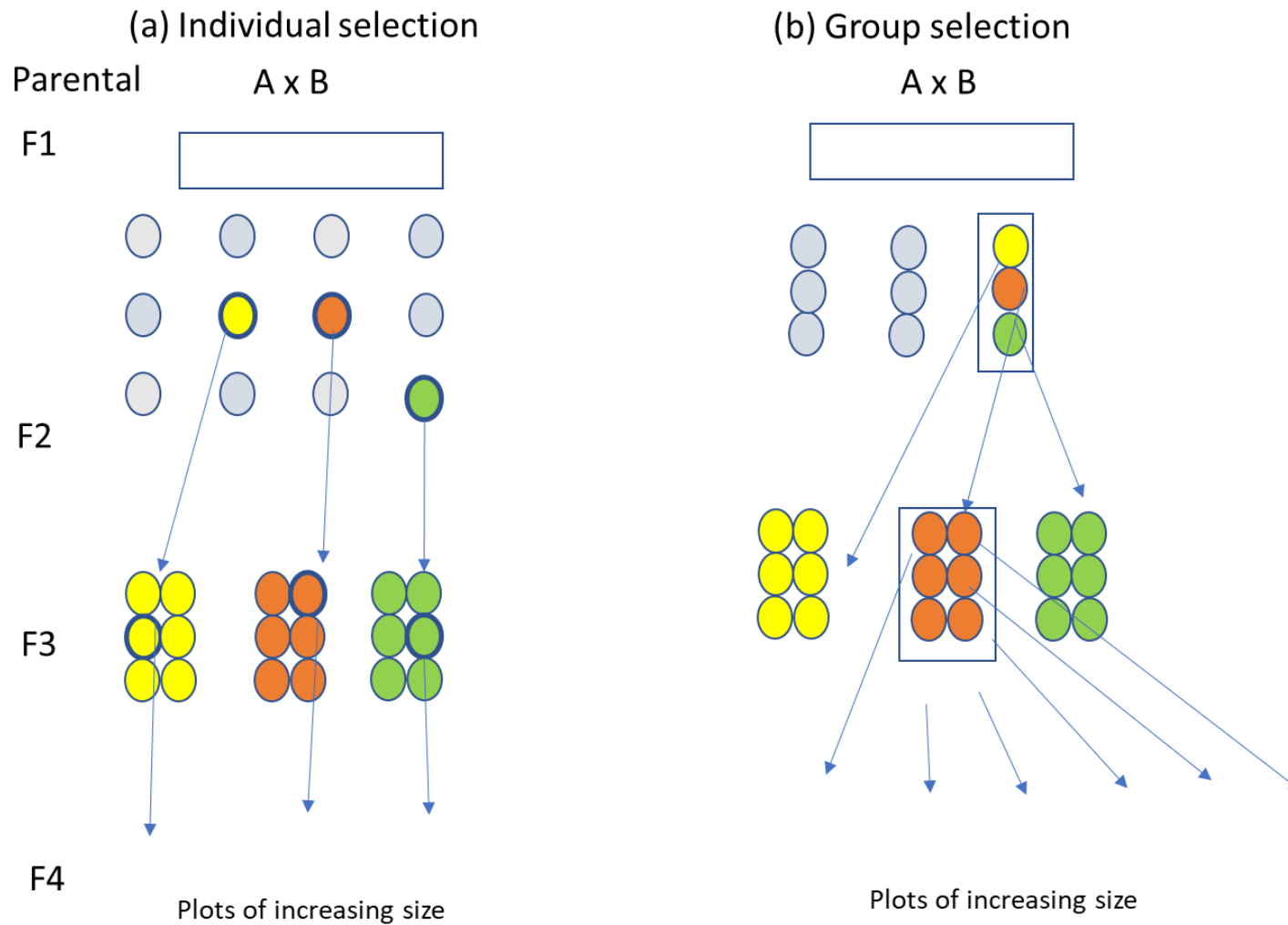


Figure 4 Schematic depiction of a phenotypic selection program from an inbreeding crop following a cross between two homozygous parents producing heterozygous offspring based predominantly on (a) individual and (b) group selection. In (a) the F1 generation are selfed and selection

starts in the F2 in bulk plots. The highest yielding individuals (colored circles) are selected as parents for F3 and grown in small plots. From each these plots again the best performing individuals (bold circles) are selected to tested on larger and more farm like conditions. In (b) the F2 are grown in groups, from the best performing group (indicated by the vertical rectangle) all individuals are selected as parents for the F3 generation, also grown in small plots. All individual from the best performing group are selected for further testing. Evidently (a) allows for a larger number of genotypes to be tested in a limited space whereas (b) more directly selects on group performance and more likely selects for kin recognition. (Modified from Murphy et al. 2017).

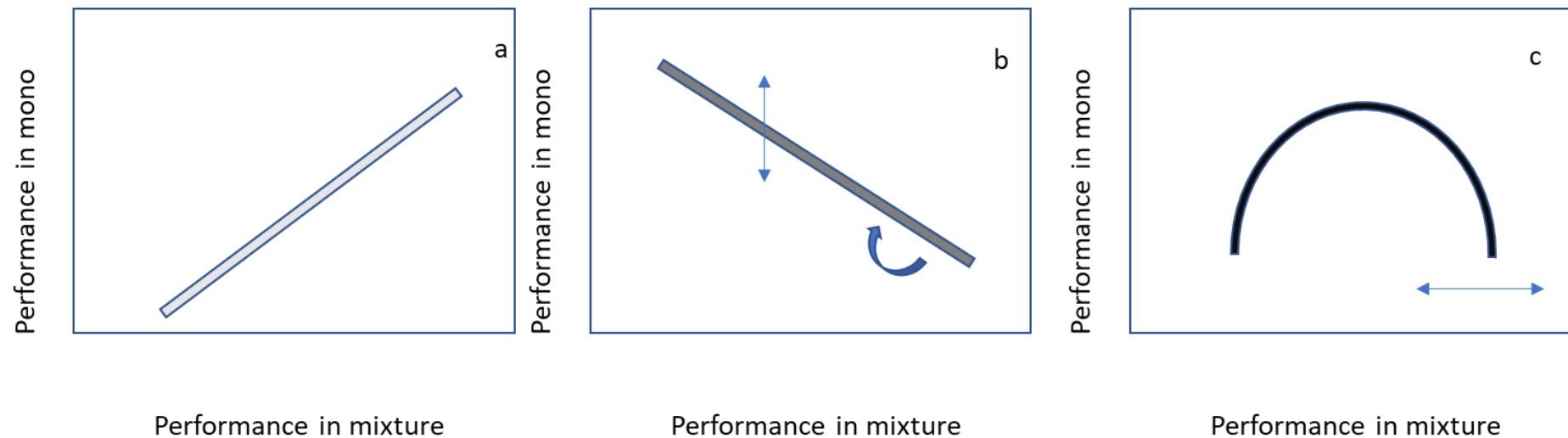


Figure 5 Three hypothetical relationships between group performance in monostands (performance of a stand of genetically [almost] identical plants) and individual performance in mixtures (how well does a plant do when competing with genetically distinct neighbors). In (a) there is positive relationship entailing that selection for individual performance in mixtures would also result in high group performance, and group selection in crop breeding is not needed. In (b) there is a negative correlation, selecting the best performer in a mixtures results in the worst group performer and group selection is an option. The role of kin recognition in enhancing crop production depends on (i) the steepness of this tradeoff (the double arrow) and (ii) the effect of kin recognition along this trade off (curved arrow). In reality the relationship is often mixed (c) (see Weiner et al. 2017) and benefits of seeking kin recognition through group selection may depend on where a population falls along this relationship.