

IS KIN RECOGNITION IN *CAKILE EDENTULA* AFFECTED BY NUTRIENT
AVAILABILITY?

IS KIN RECOGNITION IN *CAKILE EDENTULA* AFFECTED BY NUTRIENT
AVAILABILITY?

BY

MUDRA V. BHATT, B.Sc.

A Thesis

Submitted to the School of Graduate Studies
in Partial Fulfilment of the Requirements for the Degree
Master of Science

MASTER OF SCIENCE (2013)

McMaster University

(Biology)

Hamilton, Ontario

TITLE: Is kin recognition in *cahile edentula* affected by nutrient availability?

AUTHOR: Mudra V. Bhatt

SUPERVISOR: Dr. Susan A. Dudley

NUMBER OF PAGES: xiv, 92

ABSTRACT

As plants are sessile organisms, detecting the presence of neighboring plants and exhibiting competitive behavior to acquire limiting resources is crucial. One of the ways plants respond to belowground competition is by allocation to fine roots in order to acquire the limited resources. However, this phenotypic plasticity can be costly as it assigns resources away from reproduction. Being able to recognize the relatedness of one's neighbours and preferentially compete with strangers is a beneficial trait that can minimize the costs of competition with relatives and increases inclusive fitness. Many studies have looked at the association between resource availability and competition in plants while others have observed kin recognition in several plants species. However, no one has yet studied the effect of resource availability on kin recognition in plants. Here, I looked at root architecture to test if there is an association between kin recognition and nutrient availability in *Cakile edentula*.

I found that the root system architecture is highly plastic and complex, showing variable responses to neighbour identity signals and resource availability. The results from the four experiments demonstrate that the responses of *C. edentula* to neighbour relatedness are dependent on nutrient availability. Additionally, this study also indicates that kin recognition in *C. edentula* does not require root contact; instead it occurs through a signal found in soluble compounds excreted from plants, possibly root exudates, as observed in *Arabidopsis thaliana* (Biedrzycki et al. 2010).

In conclusion, this study provides novel findings regarding the dynamics of root behavior in response to nutrient availability and the relatedness of neighbours.

ACKNOWLEDGEMENTS

I would foremost like to thank my supervisor, Dr. Susan A. Dudley, for her continuous patience, guidance and support. Next, I would like to thank my committee members, Dr. Robin Cameron and Dr. Harsh Bais, for their helpful advices. I wish to thank Amanda File and Guillermo Murphy for being my academic siblings and creating a great work environment; Patrick Langille and Alexandra Jennings for their assistance in harvesting. Finally, I would like to thank my parents and Vrushank Lakhia for all the support and encouragement.

TABLE OF CONTENTS

Abstract.....	iii
Acknowledgements.....	v
Table of Contents.....	vi
List of Figures.....	viii
List of Tables.....	xi
Chapter 1: Introduction.....	1
1.1 Aboveground and belowground competition through phenotypic plasticity.....	1
1.2 Kin recognition in plants.....	3
1.3 Root exudates as the basis for kin recognition in plants.....	5
1.4 Effect of Resource Limitation on Kin Recognition.....	6
1.5 Root architecture.....	8
1.6 Outline of this study.....	10
Chapter 2: Methods.....	12
2.1 Study species.....	12
2.2 Experimental design.....	12
2.3 Data collection.....	16
2.4 Data analysis.....	17
Chapter 3: Results.....	25
3.1 No- nutrient experiment.....	25
3.2 Nutrient-rich experiment.....	36

3.3 Nutrient / no-nutrient combined experiment with four families.....	49
3.4 Nutrient / no-nutrient combined experiment with two families.....	67
Chapter 4: Discussion.....	81
References.....	88

List of Figures

Figure 2.1: A, B and C represent the experimental setup for experiment 1. The arrows show the transfer of seedlings, each day for 14 days, for Stranger, Kin and Own social environment treatment grown in sterile water. The seedlings were not randomized within the well-plates - 18

Figure 2.2: A, B and C represent the experimental setup for experiment 2, 3 and 4. The arrows show the transfer of seedlings, each day for 14 days for experiment 2 and 3 and 7 days for experiment 4, for Stranger, Kin and Own social environment treatment grown in sterile water or liquid MS media. The social environment was randomized within the nutrient or no-nutrient well-plates. - 20

Figure 2.3: Summary of variables used in data collection and data analysis for all experiments. During the harvest, length of the tap root, number of lateral roots, length of each lateral root, the hypocotyl length, and number of leaves were measured for each seedling. Once the plants were oven dried for 24 hours, total root mass, hypocotyl mass, cotyledon mass, and leaf mass was measured. Aboveground mass comprises of hypocotyl mass, cotyledon mass, and leaf mass. Total lateral length was calculated, as the sum of lateral lengths. - 22

Figure 3.1a: Effect of social environment and family on number of laterals for *Cakile edentula* seedlings grown in sterile water in well-plates switched with either kin or stranger seedling for 14 days. The letters indicate statistically significant differences between the social environment treatments at the 0.05 level. The bars indicate ± 1 standard error. - 32

Figure 3.1b: Bar graph of root allocation (least square means of root biomass with aboveground biomass as the covariate) for *Cakile edentula* seedlings, grown in sterile water in well-plates switched with either kin or stranger seedling for 14 days, in response to social environment. The letters indicate statistically significant differences between the social environment treatments at the 0.05 level. The bars indicate ± 1 standard error. - 34

Figure 3.2a: Bar graph of root elongation (least square means of tap-root length with root biomass as the covariate) for *Cakile edentula* seedlings, grown in liquid MS media in well-plates, switched with either kin or stranger seedling for 14 days, in response to social environment. The letters indicate statistically significant differences between the social environment treatments at the 0.05 level. The bars indicate ± 1 standard error. - 43

Figure 3.2b: Bar graph of root allocation (least square means of root mass with aboveground biomass as the covariate) for *Cakile edentula* seedlings, grown in liquid MS media in well-plates switched with either kin or stranger seedling for 14 days, in response to social environment. The letters indicate statistically significant differences between the social environment treatments at the 0.05 level. The bars indicate ± 1 standard error. - 45

Figure 3.2c: Bar graph of specific root length (least square means of total root length with root biomass as the covariate) for *Cakile edentula* seedlings, grown in liquid MS media in well-plates switched with either kin or stranger seedling for 14 days, in response to social environment. The letters indicate statistically significant differences between the social environment treatments at the 0.05 level. The bars indicate ± 1 standard error. - 47

Figure 3.3a: Effect of social environment and nutrient environment on tap-root length for *Cakile edentula* seedlings grown either sterile water or liquid MS media in well-plates and switched with either kin or stranger seedling for 14 days. The bars indicate ± 1 standard error. - 57

Figure 3.3b: Effect of social environment and nutrient environment on root elongation (least square means of tap-root length with root biomass as the covariate) for *Cakile edentula* seedlings grown in either sterile water or liquid MS media in well-plates and switched with either kin or stranger seedling for 14 days. The bars indicate ± 1 standard error. - 59

Figure 3.3c: Effect of social environment and nutrient environment on specific root length (least square means of total root length with root biomass as the covariate) for *Cakile edentula* seedlings grown in either sterile water or liquid MS media in well-plates and switched with either kin or stranger seedling for 14 days. The bars indicate ± 1 standard error. - 61

Figure 3.3d: Bar graph showing the effect of social environment on root allocation (least square means of root mass with aboveground biomass as the covariate) for *Cakile edentula* seedlings, grown in either sterile water or liquid MS media in well-plates switched with either kin or stranger seedling for 14 days, in response to social environment. The letters indicate statistically significant differences between the social environment treatments at the 0.05 level. The bars indicate ± 1 standard error. - 63

Figure 3.3e: Bar graph showing the effect of nutrient environment on root allocation (least square means of root mass with aboveground biomass as the covariate) for *Cakile*

edentula seedlings, grown in either sterile water or liquid MS media in well-plates switched with either kin or stranger seedling for 14 days, in response to social environment. The letters indicate statistically significant differences between the social environment treatments at the 0.05 level. The bars indicate ± 1 standard error. - 65

Figure 3.4a: Effect of social environment and nutrient environment on root elongation (least square means of tap-root length with root biomass as the covariate) for *Cakile edentula* seedlings grown in either sterile water or liquid MS media in well-plates and switched with either kin or stranger seedling for 7 days. The bars indicate ± 1 standard error. - 75

Figure 3.4b: Effect of social environment and nutrient environment on specific root length (least square means of total root length with root biomass as the covariate) for *Cakile edentula* seedlings grown in either sterile water or liquid MS media in well-plates and switched with either kin or stranger seedling for 7 days. The bars indicate ± 1 standard error. - 77

Figure 3.4c: Effect of social environment and nutrient environment on root allocation (least square means of total root mass with aboveground biomass as the covariate) for *Cakile edentula* seedlings grown in either sterile water or liquid MS media in well-plates and switched with either kin or stranger seedling for 7 days. The bars indicate ± 1 standard error. - 79

Figure 4.1: Summary of the hypothesized complex decisions required in producing and recognizing identity signals. The lack of kin recognition response in root allocation in seedlings grown in the nutrient-deprived environment could be a result of an error in any of these three steps. - 86

List of Tables:

Table 2.1: Summary of the ingredients present in Murashige and Skoog Basal Medium with vitamins that was used as the nutrient solution in all four experiments. - 24

Table 3.1a: Analysis of variance for root architecture of *Cakile edentula* seedlings grown in sterile water in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 87. N = 95. Family is A, B, or C; Social environment is kin, stranger or own exudates. - 26

Table 3.1b: Analysis of variance for aboveground trait of *Cakile edentula* seedlings grown in sterile water in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 87. N = 95. Family is A, B, or C; Social environment is kin, stranger or own exudates. Overall model significance is $P < 0.1273$ - 27

Table 3.1c: Analysis of variance for dry biomass of *Cakile edentula* seedlings grown in sterile water in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 87. N= 95. Family is A, B, or C; Social environment is kin, stranger or own exudates. - 28

Table 3.1d: Root elongation analysis from analysis of covariance of tap-root length as the dependent variable and root mass as the covariate for *Cakile edentula* seedlings grown in sterile water in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 86. N=95. Overall model significance is $P < 0.0003$. - 29

Table 3.1e: Root allocation analysis from analysis of covariance of root mass as the dependent variable and aboveground mass as the covariate for *Cakile edentula* seedlings grown in sterile water in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 86. N=95. Overall model significance is $P < 0.0256$. - 30

Table 3.1f: Specific root length from analysis of covariance of total root length as the dependent variable and root mass as the covariate for *Cakile edentula* seedlings grown in sterile water in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 86. N=95. Overall model significance is $P < 0.0025$. - 31

Table 3.2a: Analysis of variance for root architecture traits of *Cakile edentula* seedlings grown in liquid MS media in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 94. N = 105. Family is C, F, G or H; Social environment is kin, stranger or own exudates. - 37

Table 3.2b: Analysis of variance for aboveground trait of *Cakile edentula* seedlings grown in sterile liquid MS media in well-plates switched with either kin or stranger

seedling for 14 days. Error d.f. is 87. N = 95. Family is C, F, G or H; Social environment is kin, stranger or own exudates. Overall model significance is $P < 0.0927$. - 38

Table 3.2c: Analysis of variance for dry biomass of *Cakile edentula* seedlings grown in liquid MS media in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 94. N = 105. Family is C, F, G or H; Social environment is kin, stranger or own exudates. - 39

Table 3.2d: Root elongation analysis from analysis of covariance of tap-root length as the dependent variable and root mass as the covariate for *Cakile edentula* seedlings grown in liquid MS media in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 92. N = 104. Overall model significance is $P < 0.0089$. - 40

Table 3.2e: Root allocation analysis from analysis of covariance of root mass as the dependent variable and aboveground mass as the covariate for *Cakile edentula* seedlings grown in liquid MS media in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 92. N = 104. Overall model significance is $P < 0.0001$. - 41

Table 3.2f: Specific root length from analysis of covariance of total root length as the dependent variable and root mass as the covariate for *Cakile edentula* seedlings grown in liquid MS media in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 92. N = 104. Overall model significance is $P < 0.0060$. - 42

Table 3.3a: Analysis of variance for root architecture traits of *Cakile edentula* seedlings grown in liquid MS media or sterile water in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 228. N = 251. Family is C, D, B, E; Social environment is kin, stranger or own exudates; nutrient environment is nutrient and no nutrient. - 51

Table 3.3b: Analysis of variance for hypocotyl length of *Cakile edentula* seedlings grown in sterile liquid MS media or sterile water in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 228. N = 251. Family is C, D, B, E; Social environment is kin, stranger or own exudates; Nutrient environment is nutrient and no nutrient. - 52

Table 3.3c: Analysis of variance for dry biomass of *Cakile edentula* seedlings grown in liquid MS media or sterile water in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 228. N = 251. Family is C, D, B, E; Social environment is kin, stranger or own exudates; Nutrient environment is nutrient and no nutrient. - 53

Table 3.3d: Root elongation analysis from analysis of covariance of tap-root length as the dependent variable and root mass as the covariate for *Cakile edentula* seedlings grown in liquid MS media or sterile water in well-plates switched with either kin or stranger seedling for 14 days. Family is C, D, B, E; Social environment is kin, stranger or own exudates; Nutrient environment is nutrient and no nutrient. Error d.f. is 226. N=250. Overall model significance is $P < 0.0001$. - 54

Table 3.3e: Root allocation analysis from analysis of covariance of root mass as the dependent variable and aboveground mass as the covariate for *Cakile edentula* seedlings grown in liquid MS media or sterile water in well-plates switched with either kin or stranger seedling for 14 days. Family is C, D, B, E; Social environment is kin, stranger or own exudates; Nutrient environment is nutrient and no nutrient. Error d.f. is 226. N=250. Overall model significance is $P < 0.0001$. - 55

Table 3.3f: Specific root length from analysis of covariance of total root length as the dependent variable and root mass as the covariate for *Cakile edentula* seedlings grown in liquid MS media in well-plates switched with either kin or stranger seedling for 14 days. Family is C, D, B, E; Social environment is kin, stranger or own exudates; Nutrient environment is nutrient and no nutrient. Error d.f. is 226. N=250. Overall model significance is $P < 0.0001$. - 56

Table 3.4a: Analysis of variance for root architecture traits of *Cakile edentula* seedlings grown in liquid MS media or sterile water in well-plates switched with either kin or stranger seedling for 7 days. Error d.f. is 166. N = 177. Family is C or E; Social environment is kin, stranger or own exudates; nutrient environment is nutrient and no nutrient. - 69

Table 3.4b: Analysis of variance for aboveground trait of *Cakile edentula* seedlings grown in sterile liquid MS media or sterile water in well-plates switched with either kin or stranger seedling for 7 days. Error d.f. is 166. N = 177. Family is C or E; Social environment is kin, stranger or own exudates; nutrient environment is nutrient and no nutrient. -70

Table 3.4c: Analysis of variance for dry biomass of *Cakile edentula* seedlings grown in liquid MS media or sterile water in well-plates switched with either kin or stranger seedling for 7 days. Error d.f. is 166. N = 177. Family is C or E; Social environment is kin, stranger or own exudates; nutrient environment is nutrient and no nutrient. - 71

Table 3.4d: Root elongation analysis from analysis of covariance of tap-root length as the dependent variable and root mass as the covariate for *Cakile edentula* seedlings grown in

liquid MS media or sterile water in well-plates switched with either kin or stranger seedling for 7 days. Error d.f. is 165. N = 177; Family is C or E; Social environment is kin, stranger or own exudates; Nutrient environment is nutrient and no nutrient. Overall model significance is $P < 0.0001$. - 72

Table 3.4e: Root allocation analysis from analysis of covariance of root mass as the dependent variable and aboveground mass as the covariate for *Cakile edentula* seedlings grown in liquid MS media or sterile water in well-plates switched with either kin or stranger seedling for 7 days. Error d.f. is 166. N=177; Family is C or E; Social environment is kin, stranger or own exudates; Nutrient environment is nutrient and no nutrient. Overall model significance is $P < 0.0001$. - 73

Table 3.4f: Specific root length from analysis of covariance of total root length as the dependent variable and root mass as the covariate for *Cakile edentula* seedlings grown in liquid MS media in well-plates switched with either kin or stranger seedling for 7 days. Error d.f. is 165. N=177. Family is C or E; Social environment is kin, stranger or own exudates; nutrient environment is nutrient and no nutrient. Overall model significance is $P < 0.0001$. - 74

CHAPTER 1: INTRODUCTION

1.1 Aboveground and belowground competition through phenotypic plasticity

Competition is one of the most important social interactions for plants. Plants can detect the presence of neighbouring plants (Novoplansky 2009) using both above- and below-ground cues and respond by exhibiting competitive behavior (Callaway 2002) to acquire scarce resources such as light, water and nutrients. As sessile organisms, plants utilize phenotypic plasticity, the ability to change phenotype in response to an environmental cue, to become better competitors (Trewavas 2009) and to better acquire limiting resources.

Light is a key and, in many cases, a limiting energy resource for plants. As sessile photoautotrophs, plants actively adjust their growth and positioning of leaves to acquire optimal light energy for photosynthesis. In addition to providing energy for photosynthesis, light signals also provide plants with important spatial and temporal information about their surrounding environment. Light signals are perceived by photoreceptors that contain pigments specialized in absorbing light from specific parts of the light spectrum. These specialized information-transducing photoreceptors include red (R) and far-red (FR) light- absorbing phytochromes and blue (UV-A) light- absorbing receptors (Smith 1982). When daylight passes through vegetation, chlorophyll and carotenoid pigments within the leaves preferentially absorb red (R) and blue (B) wavelengths and reflect back far-red (FR) wavelengths. A high density vegetation results in light that is rich in far-red (FR) and poor in red (R) and blue (B) wavelengths (Franklin and Whitelam 2005). Hence, a decreased red:far red light ratio (R:FR) acts as an

aboveground cue that tells the plant that it is being shaded or has aboveground neighbours present (Smith 1982). The phytochromes sense this change in light and can respond to this by allocating more biomass to the aboveground organs (Whitelam and Smith 1991) or elongating aboveground organs, including hypocotyl and petiole elongation, and upward positioning of leaves (hyponasty) (Ballare, Scopel et al. 1990) . When light is the limiting resource, this type of phenotypic plasticity in the presence of neighbours allows the individual to become taller than its neighbours and capture more light.

However, much of the competition in plants takes place underground. There are multiple resources found in soil, in contrast to a single key resource found aboveground (Casper and Jackson 1997). Plants actively compete with other neighbouring plants for belowground resources, which include water and around 20 essential mineral nutrients that differ in molecular size, oxidation state, and mobility within the soil (Casper and Jackson 1997).

Plants can increase root proliferation in regions with high nutrient concentrations as a mechanism to exploit resource patches when under competitive situations (Hodge 2009); (de Kroon and Mommer 2006). An individual's competitive ability is greatly dependent on root density, surface area and the plasticity in root growth (Casper and Jackson 1997). Several studies have shown that, in the presence of belowground neighbours, plants increase root proliferation even before nutrients are diminished, suggesting that plants can show plasticity to roots of neighbours and not just to resource depletion (Gersani, Brown et al. 2001, Maina, Brown et al. 2002, O'Brien, Gersani et al. 2005). In these cases, plants can predict the impending root competition and respond by

increasing biomass allocation to the fine roots to increase their competitive ability (Gersani, Abramsky et al. 1998, Maina, Brown et al. 2002, Falik, Reides et al. 2003, Murphy and Dudley 2007). However, this type of phenotypic plasticity is costly as it assigns resources away from reproduction (DeWitt, 1998).

Mahal and Callaway (1991) were the first to provide the initial evidence for self /non-self recognition by roots in the desert shrub *Ambrosia dumosa*. Individuals from this species continue normal root growth when they encounter their own roots but stop root growth when they encounter roots from another *A. dumosa* plant. Thereafter, studies with soybean (Gersani, Brown et al. 2001), strawberry (Holzapfel and Alpert 2003), peas (Falik, Reides et al. 2003), buffalo grass (Gruntman and Novoplansky 2004), and Kenya bean (Maina, Brown et al. 2002) also provided evidence for self / non-self recognition at the root level. However, these studies showed that plants allocate more biomass to roots when they encounter a non-self neighbour compared to when they are grown alone. Subsequent root identity recognition studies have shown that plants can also distinguish and respond to species (Huber-Sannwald, Pyke et al. 1996), and most interestingly, relatedness (Dudley (Dudley and File 2007, Murphy and Dudley 2009, Bhatt, Khandelwal et al. 2010, Biedrzycki, Jilany et al. 2010) of the neighboring roots.

1.2 Kin recognition in plants

Kin recognition, an ability to distinguish between relatives and non-relatives, is a beneficial social trait when living in dense related groups, allowing individuals to direct altruistic behaviours towards individuals with shared alleles, thus increasing the indirect fitness of the focal individual (Waldman 1988). Plant life histories often result in mono-

specific stands of closely related individuals, creating conditions ideal for kin selection (Hamilton 1964, Waldman 1988, Kelly 1996). Donohue (2003) showed that individuals of *Cakile edentula* had higher fitness when growing in groups of siblings compared to groups of strangers. Therefore, like animals, it would be beneficial for plants to have the ability to discriminate between relatives and non-relatives and only compete with non-related (stranger) neighbours. This would reduce the cost of competition and increase indirect fitness. So far plant kin recognition has been observed in *Cakile edentula* (Dudley and File 2007, Murphy and Dudley 2009, Bhatt, Khandelwal et al. 2010), *Impatiens pallida* (Murphy and Dudley 2009), *Chenopodium album* (Dudley et al. unpublished data), and *Arabidopsis thaliana* (Biedrzycki, Jilany et al. 2010). All four studies observed differential competitive behaviour to siblings compared to strangers, which is consistent with kin selection. *I. pallida* and *C. album* show increased competitive response, in aboveground traits, towards non-related conspecific individuals. *I. pallida*, a species that is found in woody areas where light is a limiting resource, allocated more resources to leaves, in relation to roots, when grown with non-related plants and changed their aboveground morphology in presence of siblings to reduce competition (Murphy and Dudley 2009). Similarly, *C. album*, a weedy annual plant usually found in crop fields, also allocated more resources to aboveground traits, in relation to roots, when grown with non-related plants (Dudley, Lee et al. unpublished data). Conversely, *C. edentula* and *A. thaliana*, usually found in areas with limiting belowground resources, showed an increased competitive response, in belowground traits, towards non-related conspecific individuals than related individuals. *C. edentula* plants showed increased root allocation

in relation to aboveground traits and *A. thaliana* showed increased number of lateral roots when interacting with non-related individuals (Dudley and File 2007, Bhatt, Khandelwal et al. 2010, Biedrzycki, Jilany et al. 2010). The competitive responses of each species, in the presence of a non-relative, are consistent with their native ecology. However, they all require belowground cues (i.e. root interaction) for this kin recognition to occur (Karban, 2010).

1.3 Root exudates as the basis for kin recognition in plants

There is evidence that root exudates are the basis for the kin recognition response in plants (Bais, Park et al. 2004). Plants lose as much as 5-21% of their carbon into soil in a form of root exudates (Badri, Weir et al. 2009). Root exudates consist of soluble molecules consisting of ions, free oxygen, water, enzymes, mucilage, primary and secondary metabolites, exuded by the plant roots (Bais, Weir et al. 2006). Root exudates have various ecological and biochemical functions and influence plant-microbial and plant-plant interactions, nutrient and water uptake, and detoxification of toxins (Bais, Weir et al. 2006). Research has shown that roots exude an array of compounds in response to stress and the exudate profile may vary depending on the biological or chemical elicitors (Walker, Bais et al. 2003). It has been hypothesized that plants can use root exudates as a source of information about neighbouring plants as the combination of secondary metabolites secreted by the roots are species specific (Flores, Vivanco et al. 1999, Bais, Loyola-Vargas et al. 2001). Biedrzycki et al. (2010) tested whether root exudates were a source of information about relatedness by growing *A. thaliana* seedlings in well-plates and exposing them to liquid media in which either a kin, stranger, or itself

was growing, without any root contact. The study looked at the response of seedlings grown with sodium orthovanadate, a secretion transport inhibitor, exposed to liquid media in which either a kin, stranger, itself was growing. Their results showed that the kin recognition response disappeared when the roots were treated with sodium orthovanadate. This provided evidence for root exudates as the basis of kin recognition in *A. thaliana* (Biedrzycki, Jilany et al. 2010).

1.4 Effect of Resource Limitation on Kin Recognition

Competition among animals for limited resources plays an important role in shaping life-history strategies and evolution of behaviour (Lomnicki 1988). There are two main behavioural strategies observed in animal behaviour studies.

The first strategy follows the kin selection theory of altruism, which states that individuals will direct competition away from relatives, even at a cost to themselves to increase their indirect fitness (Hamilton 1964). According to Hamilton's rule, altruistic behavior among animals will prevail only if the indirect fitness benefit (i.e. helping relatives) to the donor is greater than the cost of performing an altruistic act. Hamilton's rule can be simply explained by $r \cdot B > C$, where r is the genetic relatedness between the donor and the recipient, B is the fitness benefit to recipient and C is the cost to the donor (Hamilton 1964). As resources become limiting, an individual may be more inclined to accept the cost of direct fitness to favour the reproductive success of their relatives, in order to increase its inclusive fitness (Hamilton 1964). There are a lot of animal behaviour examples that support this theory. One good example to support this theory comes from observing the den sharing behaviour of Australian mountain brushtail

possums (Banks, Lindenmayer et al. 2011). Banks *et al.* (2011) looked at the effect of resource limitation on kin selection in Australian brushtail possums, whose key resource is hollow trees for dens. Changes in den sharing patterns indicate of a decline in the key resource. Banks (2011) observed that hollow-dependent Australian mountain brushtail possums switch den-sharing preference from kin avoidance to kin preference with decreasing tree hollow availability. This suggests that with limiting resources, Australian brushtail possums are more inclined to accept the cost of inbreeding, in order to increase survival chances of a relative.

The second strategy is usually observed in animals if the competition is occurring in a closed system, where the competitors (related and non-related conspecifics) cannot leave an area and are competing for the same resources. In this case, the cost of altruistic behaviour increases as the occurrence of competition with kin members increase (West and Gardner 2010). Hence, in this scenario, animals are more likely to act selfishly by not discriminating between kin and stranger and competing equally with both conspecifics, as the key resource decreases. One good example of this theory comes from observing the tadpoles of Spadefoot toads, *Scaphiopus bombifrons*. Spadefoot toads are native to North America and live in dry, desert areas. These small toads breed once a year in small temporary ponds formed by the rain (Pfennig, Reeve et al. 1993), and the tadpoles spend their early life in the small pond making this a closed system. Pfennig *et al.* (1993) examined the carnivore morph of Spadefoot toads to determine if it differentiates between kin and non-kin and if this behaviour is context- dependent. The study showed that, in normal circumstances, Spadefoot toads use chemical cues to discriminate kin and avoid

eating siblings to increase their inclusive fitness. However, when hungry, tadpoles of Spadefoot toads become less selective in their prey choice and preyed on kin just as much as non-kin (Pfennig, Reeve et al. 1993). This provides evidence for the hypothesis that the altruistic behaviour will become less prevalent as resource availability decreases.

As plants are sessile organisms, they cannot move from an area to area looking for high resource patches. Thus, exploiting limiting resource patches under competitive situations becomes vital for survival. Plants may be growing in groups of kin or strangers depending on the ecology and the dispersal patterns of the species and can employ phenotypic plasticity in root proliferations to forage and compete for patchy nutrient reservoirs in its surrounding area. *Cakile edentula* increased competitive response when grown with strangers. However, no one has looked at the significance of resource availability on the responses to the relatedness of neighbours.

1.5 Root architecture

Within a species, root system morphology can vary greatly as a consequence of the interactions between developmental processes and environmental conditions such as soil strength, nutrient, water, and oxygen availability (Lynch 1995). As a whole, the spatial configuration of the root system shows the ability of a plant to proliferate their roots to explore and exploit below-ground resources and provide structural support. Root proliferation is a product that requires a range of morphological changes. These changes include elongation of individual roots (Jackson and Caldwell 1989), an increase in total root length (Hodge, Stewart et al. 2000), root production (Hodge, Stewart et al. 2000) and

initiation of new laterals (Farley and Fitter 1999). The direction and the extent of root system is highly influenced by environmental cues as the roots are solely responsible for the acquisition of all belowground resources (water and nutrients) (Grime, Crick et al. 1986). This spatial configuration (shape and structure) of the root system is termed root architecture. Having the correct root architecture is crucial to compete efficiently for resources in a nutrient deficient environment.

Root biomass is a commonly used measurement to study the foraging ability of plants. However, Hodge (2004) states that root biomass may not be the best measurement as changes in root system architecture can occur without a change in root biomass. Hence, studying root architecture is critical to fully understand plant root behaviour. Plants can optimize their root architecture by influencing the growth of primary and lateral roots (Malamy 2005). Root architecture can be quantified by measuring variables such as lateral root lengths, root depth, specific root length and root length density (Hodge 2009). Root length acts as a measure of root surface area and has shown to be a good predictor of nutrient capture (Hodge 2009). Root depth is a measure of how the root biomass is allocated vertically through soil and provides information about where each individual plant root will obtain water and nutrient resources, including the probability of belowground competition between neighbouring individuals (Cornelissen, Lavorel et al. 2003). Specific root length (SRL) is the ratio of root length to mass and it is a good measure of the amount of absorptive tissue per unit mass and acts as an indicator of water and nutrient acquisition (Barber and Silberbush 1984, Fitter 1985). Plants with higher

SRL form longer roots for a given dry mass investment by making root of thinner diameter (low tissue density) (Eissenstat 1991).

Many studies have used plant root architecture as a tool to study the root response to root neighbours, specific nutrients and nutrient patches (Hodge 2004). Hence, I wish to use root architecture as a tool to understand the effect of resource availability on kin recognition response in plants.

1.6 Outline of this study

Cakile edentula (Bigelow) Hook. ssp. *edentula* var. *lacustris* (Fernald)(Great Lakes sea rocket) is a highly selfing annual beach plant found in high-light and low-water and -nutrient environments around great lakes. *C. edentula* produces dimorphic seeds (distal and proximal), which have different dispersal patterns (Rodman 1974). The proximal seeds are usually buried with the mother plant and emerge in high-density sibling groups (Rodman 1974, Keddy 1982) and the distal seeds are usually dispersed away from the mother plant by wind or water creating high-density stranger groups (Payne and Maun 1981, Donohue 1998). *C. edentula* allocates less biomass to roots when grown with siblings (Dudley and File 2007, Bhatt, Khandelwal et al. 2010) suggesting kin recognition response. Additionally, studies have shown that kin groups have higher fitness compared to stranger groups (Donohue 2003) providing opportunities for kin selection. Hence, *C. edentula* is ideal for this study.

Previous studies observing kin recognition through root allocation suggest that the mechanism of kin discrimination involved root interactions (Dudley and File 2007, Murphy and Dudley 2009) with root exudates being the basis for this root communication

in *A. thaliana* (Biedrzycki, Jilany et al. 2010). Seedlings are an ideal life stage to study responses to kin and stranger interactions in *C. edentula* as individuals in the seedling stage experience the largest number of physiological and environmental constraints and exhibit increased phenotypic plasticity towards environmental cues (Weinig and Delph 2001). So far, we have observed kin recognition response in *C. edentula* through root allocation in juvenile plants (8 weeks old) (Dudley and File 2007) and young seedlings (6 and 3 weeks old) (Bhatt, Khandelwal et al. 2010) that had root contact. However, the kin recognition response has not yet been observed in 1 and 2 week old seedlings and the role of root exudates has yet to be determined. Furthermore, the effect of nutrient availability on the kin recognition response is not yet understood.

Hence, we grew *C. edentula* seedlings in either no nutrient or high nutrient condition to test whether kin recognition observed in *C. edentula* is affected by nutrient availability. For these experiments, we used the well-plate technique developed by Biedrzycki et al (2010), which exposed the seedling to exudates of another seedling without any root contact, to test if root contact is necessary for the kin recognition response. We ask if competition with kin or stranger neighbours is affected by nutrient availability? I hypothesize that the *C. edentula* seedlings will act selfishly and compete equally with kin and stranger individuals when grown in the no nutrients environment. However, when they are grown in a nutrient rich environment, *C. edentula* seedlings will show a neighbour identity recognition response by increasing competitive behaviour when exposed to stranger exudates.

CHAPTER 2: METHODS AND MATERIALS

2.1 Study species

Cakile edentula (Bigelow) Hook. ssp. *edentula* var. *lacustris* (Fernald)(Great Lakes sea rocket) seeds used in all three experiments were from fourth generation single-seed descent lines grown in the McMaster University greenhouse and allowed to self. The original founders were collected from a population at Confederation Park, Hamilton in 2005. Because this species is highly autogamous, all seeds likely result from selfing, though some insects are present in the greenhouse.

2.2 Experimental design:

Experiment 1: No nutrients

This experiment was conducted in order to look at the effects of social environment on *C. edentula* seedlings grown without nutrients. The seedlings were either exposed to their own exudates or exudates of either a sibling seedling or a stranger seedling in well-plates. For this experiment, 3 genotypes (3 Families: ON2-5C-1 = “A”; ON2-7B-1 = “B”, ON2-17C-1= “C”) and 8 replicates of each genotype combinations for stranger and own and 4 replicates of each kin pairs were used for social environment treatment. On Nov 9, 2011, seeds were surface sterilized in family groups with a 70% ethanol solution for 1 minute, then rinsed with sterile water. The seed coat of each seed was nicked with a razorblade, to increase the chances of germination, and soaked overnight in well-plates containing sterile water. Once the seeds were imbibed for a

period of 24 hours, they had germinated. One seedling per well was placed in 6 well well-plates containing 4 ml of sterile water. On day 2, filter paper (Whatman international Ltd, Maidstone, England) was placed in each well on top of the roots to hold the seedling in place. Every day for 14 days, the seedlings were lifted out of their wells using forceps and put back in their own well (OWN treatment) or exchanged positions with the seedling in the adjacent well (KIN or STRANGER treatment). For this experiment, seedlings in the Kin treatment were not paired with another seedling so each seedling was exposed to exudates of a different kin seedling each day (Figure 1b). In contrast, seedlings in the Stranger treatment were paired so that each seedling was only exposed to exudates from one other seedling (Figure 2.1). Each well-plate contained only one social environment treatment. (Figure 1) On Nov 23, 2011, the seedlings were harvested.

Experiment 2: Nutrients

This experiment was conducted in order to observe the effects of social environment on *C. edentula* seedlings grown in a nutrient-rich environment. For this experiment, 4 genotypes (4 Families: ON2-7B-31 = “B”; ON2-17C-4 = “C”; ON2-18C-6 = “G”; ON2-20A-41 = “H”) and 8 replicates of each genotype combinations for stranger and own and 4 replicates of each kin pairs were used for social environment treatment. On Feb 2, 2012, seeds were surface sterilized in family groups with 70% ethanol solution for 2 minutes, then sterilized with the seed sterilizing solution (30% bleach solution + 10ul polysorbate surfactant (Tween 20, Fisher BioReagents) for 5 minutes. The seeds were then rinsed 5 times with sterile water. The seed coat of each seed was nicked with a sterile razorblade and soaked overnight in well-plates containing sterile water to increase the chances of

germination. Once the seeds were imbibed for 24 hours, they were placed in 6 well well-plates containing 3 ml of MS media (H₂O + Murashige and Skoog with vitamins media powder (Caisson Laboratories INC, USA) and NO sucrose), with one seedling per well. Table 2.1 shows the chemical composition of the MS media used to make the nutrient solution. Each well-plate was sealed with micropore tape to allow oxygen diffusion while keeping the inside of well-plates sterile. On day 2, filter paper (Whatman international Ltd, Maidstone, England) was placed in each well on top of the roots to hold the seedling in place. Every day for 14 days, the seedlings were lifted out of their wells using forceps and put back in their own well (OWN treatment) or exchanged positions with the seedling in the adjacent well (KIN or STRANGER treatment)(Figure 2.2). 1 ml of New MS media was added to each well every 4 days (Day 5, Day 9, Day 13). The social environment treatment was randomized within all well-plates. The young seedlings were harvested on Feb 14, 2012.

Experiment 3: No nutrients/ nutrients

This was a factorial experiment designed to test whether nutrients affect the response to the social environment. The seedlings were exposed to either their own exudates or exudates of either a sibling seedling or a stranger seedling in nutrient- rich environment or nutrient deprived environment. To carry out the experiment, 4 genotypes and 4 replicates of each genotype combinations for nutrient deprived treatment and same 4 genotypes with 6 replicated of each genotype combination for the nutrient-rich treatment were used (4 Families: ON2-7B-2 = “C”; ON2-9B-1 = “D”; ON2-6C-3 = “B”; ON2-11C-1= “E”). On May 22, 2012, seeds were sterilized and prepared as per the

methods in Experiment 2. Once the seeds were imbibed for 24 hours, they were placed in 6 well well-plates containing either 3 ml of MS media (H₂O + Murashige and Skoog with vitamins media powder and NO sucrose) or 3 ml of sterile water, with one seedling per well. Each well-plate was sealed with micropore tape to allow oxygen diffusion while keeping the inside of well-plates sterile. On day 2, filter paper (Whatman international Ltd, Maidstone, England) was placed in each well on top of the roots to hold the seedling in place. Every day for 14 days, the seedlings were lifted out of their wells using forceps and put back in their own well (OWN treatment) or exchanged positions with the seedling in the adjacent well (KIN or STRANGER treatment) (Figure 2.2). 1 ml of New MS media or 1 ml of sterile water was added to each well every 4 days (Day 5, Day 9, Day 13). The social environment treatment was randomized within nutrient and no-nutrient treatment well-plates. The seedlings were harvested on June 5, 2012

Experiment 4: No nutrients/ nutrients for 2 families

This, similarly to experiment 3, was a factorial experiment to test for an interaction between the social environment and nutrients, but here using only 2 families. The seedlings were exposed to either their own exudates or exudates of either a sibling seedling or a stranger seedling in nutrient- rich environment or nutrient deprived environment. For this experiment, 2 genotypes and 16 replicates of each genotype combinations for stranger and own and 8 replicates of each kin pairs were used for social environment treatment for each nutrient environment treatment (2 Families: ON2-7B-4= “C”; ON2-11C-1= “E”). On October 17 2012, seeds were sterilized and prepared as per the methods in Experiment 2. Once the seeds were imbibed for 24 hours, they were

placed in 6 well well-plates containing either 3 ml of MS media (H₂O + Murashige and Skoog with vitamins media powder and NO sucrose) or 3 ml of sterile water, with one seedling per well. On day 2, filter paper (Whatman international Ltd, Maidstone, England) was placed in each well on top of the roots to hold the seedling in place. Every day for 7 days, the seedlings were lifted out of their wells using forceps and put back in their own well (OWN treatment) or exchanged positions with the seedling in the adjacent well (KIN or STRANGER treatment) (Figure 2.2). 1 ml of New MS media or 1 ml of sterile water was added to each well every 2 days (Day 3, Day 5). The social environment treatment was randomized within nutrient and no-nutrient treatment well-plates. The seedlings were harvested on October 25, 2012.

2.3 Data collection

During the harvest, to quantify the root architecture, tap root length, number of laterals, and length of each lateral was measured (Figure 2.3). I also measured the hypocotyl length, and the number of. Each seedling was then separated and put into 2 separate envelopes (Staples #1 coin envelopes): 1) Aboveground biomass (hypocotyl, cotyledons, and leaves); 2) Belowground biomass (main tap root and lateral roots). All the coin envelopes with the plant parts were dried in an oven at 50 degrees Celsius for 24 hours and the dry weight of total root, hypocotyl, cotyledon, and leaves was then recorded. Each experiment was harvested on a single day. Tap root length and length of each lateral was measured with a ruler to closest 0.1mm. The number of lateral for each seedling was counted as any branch with a length of 0.1mm or longer. The dry mass of each plant part was weighed to 4 decimal places.

2.4 Data analysis

Statistical analysis of all collected data was done using SAS statistical software (version 9.2 for Windows (English); SAS Institute, Cary, NC, USA). The total lateral length is the sum of all lateral lengths and the total root length is the sum of the taproot length and total lateral length (FIGURE 3). Analyses of variance (ANOVA) and covariance (ANCOVA) were carried out with PROC GLM. ANOVA was carried out to compare means of independent groups within each dependent variable, where independent variables were family, social environment (Kin, Stranger, and Own) and nutrient environment (Nutrient and No- nutrient). Residual analysis was performed to check whether the residuals met the assumptions of the ANOVA, and, if necessary, natural logarithms (\log_e) were taken of raw data so that residuals were homoscedastic and normally distributed. Root allocation was calculated from an ANCOVA analysis using root mass as the dependant variable and aboveground mass as the covariant variable for social (kin/own/stranger) and nutrient (nutrient-rich/no-nutrient) environment treatments. Specific root length (SRL, mg^{-1}) was calculated from an ANCOVA analysis using total root length as the dependant variable and total root mass as the covariant variable for social (kin/own/stranger) and nutrient (nutrient-rich/no-nutrient) environment treatments. Root elongation was calculated from an ANCOVA analysis using taproot length as the dependant variable and total root mass as the covariant variable for social (kin/own/stranger) and nutrient (nutrient-rich/no-nutrient) environment treatments.

Figure 2.1: A, B and C represent the experimental setup for experiment 1. The arrows show the transfer of seedlings, each day for 14 days, for Stranger, Kin and Own social environment treatment grown in sterile water. The seedlings were not randomized within the well-plates.

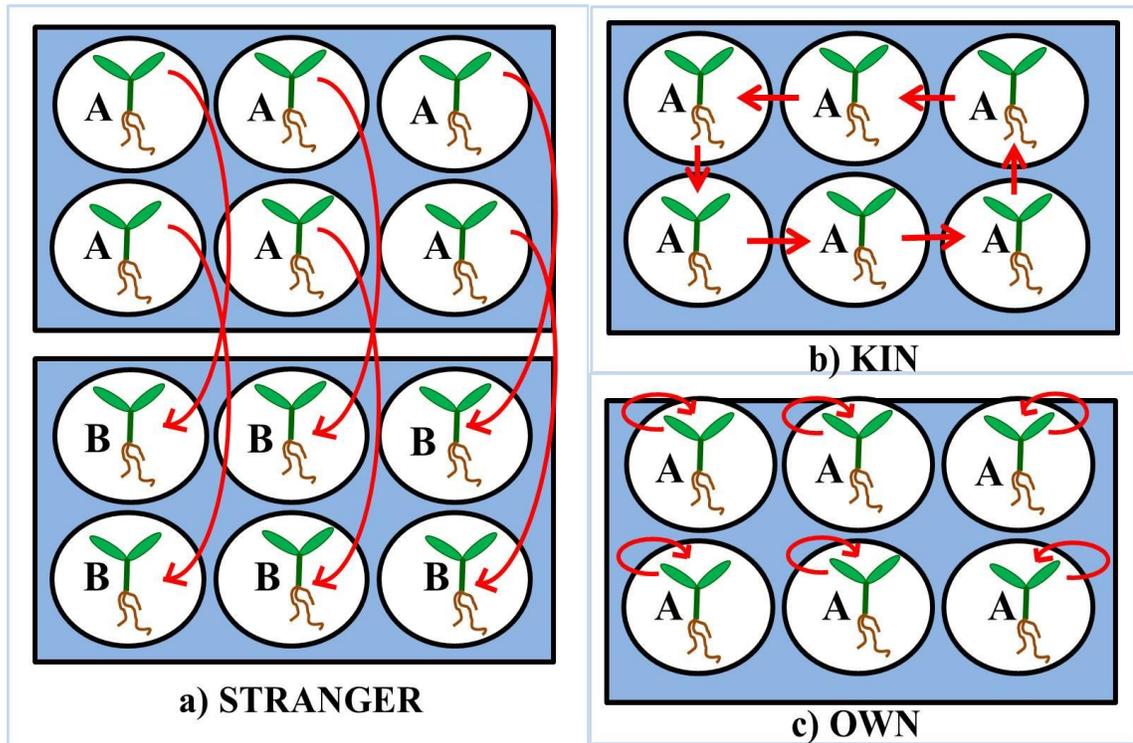


Figure 2.2: A, B and C represent the experimental setup for experiment 2, 3 and 4. The arrows show the transfer of seedlings, each day for 14 days for experiment 2 and 3 and 7 days for experiment 4, for Stranger, Kin and Own social environment treatment grown in sterile water or liquid MS media. The social environment was randomized within the nutrient or no-nutrient well-plates.

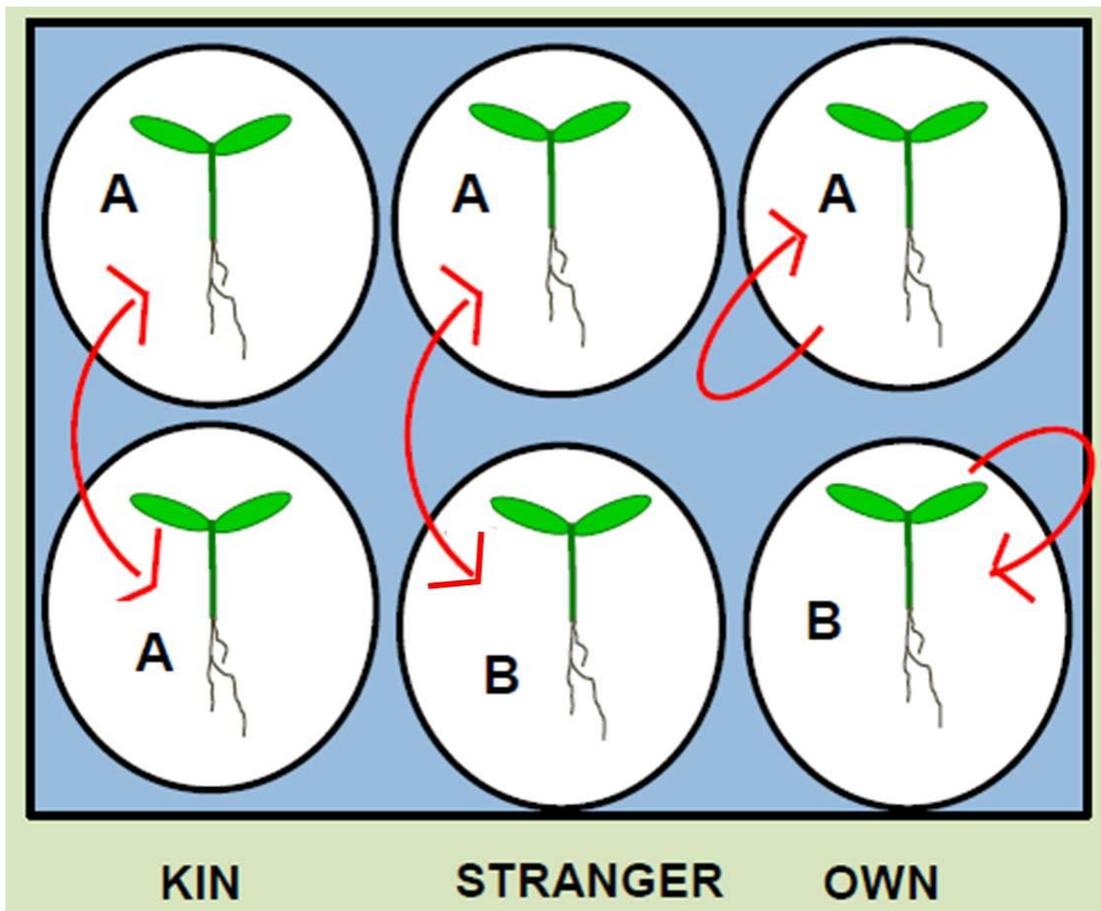


Figure 2.3: Summary of variables used in data collection and data analysis for all experiments. During the harvest, length of the tap root, number of lateral roots, length of each lateral root, the hypocotyl length, and number of leaves were measured for each seedling. Once the plants were oven dried for 24 hours, total root mass, hypocotyl mass, cotyledon mass, and leaf mass was measured. Aboveground mass comprises of hypocotyl mass, cotyledon mass, and leaf mass. Total lateral length was calculated, as the sum of lateral lengths.

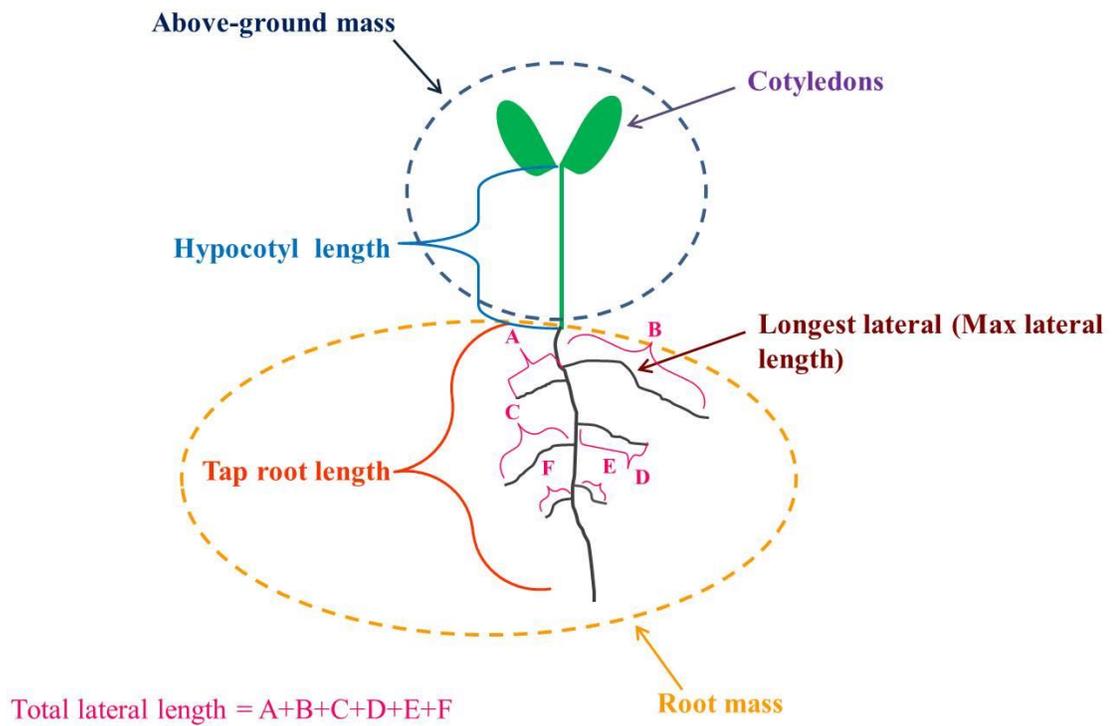


Table 2.1: Summary of the ingredients present in Murashige and Skoog Basal Medium with vitamins (Sigma-Aldrich Co. LLC.) that was used as the nutrient solution in all four experiments.

Ingredient	Percent
Ammonium Nitrate	38.19%
Boric Acid	0.14 %
Calcium Chloride anhydrous	7.69%
Cobalt Chloride • 6H ₂ O	0.0006%
Cupric Sulfate • 5H ₂ O	0.0006%
EDTA, Na ₂	0.86%
Ferrous sulfate • 7H ₂ O	0.64 %
Magnesium Sulfate	4.17 %
Manganese sulfate • H ₂ O	0.39 %
Molybdic acid (sodium salt) • 2H ₂ O	0.006%
Potassium Iodide	0.019%
Potassium Nitrate	43.96%
Potassium phosphate monobasic	3.92%

CHAPTER 3: RESULTS

3.1 No- nutrient experiment

For this experiment, seedlings from three families were grown in sterile water for 14 days. There was no significant main effect of social environment and family on tap-root length ($p < 0.1648$), \log_e total lateral length ($p < 0.0749$), hypocotyl length ($p < 0.1273$), total mass ($p < 0.0208$), or total root length ($p < 0.3094$). There were significant social environment \times family interactions for total mass ($p < 0.0118$), and aboveground mass ($p < 0.0114$). Family A and C seedlings had higher total and aboveground mass when exposed to own exudates than kin or stranger exudates. Family B seedlings had lower total and aboveground mass when exposed to own exudates than kin or stranger exudates (Table 3.1a, 3.1b, and 3.1c). There were significant main effects of social environment and family on number of laterals (Table 3.1a). Number of laterals increased in the presence of kin and stranger exudates but not in presence of own exudates. Additionally, family C produced more laterals compared to family A and B (Figure 3.1a). The root allocation patterns found in this experiment were not consistent with the kin recognition patterns previously observed in *Cakile edentula* (File 2007, Bhatt 2011). Tap- root length relative to total root mass (root elongation), allocation to roots relative to aboveground mass (root allocation), and total root length relative total root mass (specific root length) did not differ between seedlings exposed to kin or stranger exudates (Table 3.1d, 3.1e, 3.1f; Figure 3.1b).

Table 3.1a: Analysis of variance for root architecture of *Cakile edentula* seedlings grown in sterile water in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 87. N = 95. Family is A, B, or C; Social environment is kin, stranger or own exudates.

Source	d.f.	Tap-root length		ln (Total lateral length)		Number of lateral		Total root length	
		F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
Family (F)	2	1.05	0.3534	6.18	0.0031	6.87	0.0017	1.39	0.2558
Social	2	1.04	0.3586	0.07	0.9331	4.29	0.0167	0.20	0.8167
Environment (S)									
F × S	4	1.66	0.1675	1.16	0.3350	0.66	0.6190	2.06	0.0926

Table 3.1b: Analysis of variance for aboveground trait of *Cakile edentula* seedlings grown in sterile water in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 87. N = 95. Family is A, B, or C; Social environment is kin, stranger or own exudates. Overall model significance is $P < 0.1273$

Source	d.f.	Hypocotyl length	
		F-ratio	P-value
Family (F)	2	0.13	0.8795
Social Environment (S)	2	3.84	0.0253
F × S	4	1.35	0.2598

Table 3.1c: Analysis of variance for dry biomass of *Cakile edentula* seedlings grown in sterile water in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 87. N= 95. Family is A, B, or C; Social environment is kin, stranger or own exudates.

Source	d.f.	Total mass		Root mass		Aboveground mass	
		F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
Family (F)	2	0.45	0.6410	0.51	0.6020	0.40	0.6729
Social Environment (S)	2	1.78	0.1747	1.85	0.1639	1.96	0.1468
F × S	4	3.43	0.0118	0.38	0.8254	3.87	0.0061

Table 3.1d: Root elongation analysis from analysis of covariance of tap-root length as the dependent variable and root mass as the covariate for *Cakile edentula* seedlings grown in sterile water in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 86. N=95. Overall model significance is $P < 0.0003$.

Source	d.f.	Root elongation	
		F-ratio	P-value
Root mass	1	20.37	<0.0001
Family (F)	2	2.13	0.1252
Social Environment (S)	2	0.47	0.6262
F × S	4	2.04	0.0954

Table 3.1e: Root allocation analysis from analysis of covariance of root mass as the dependent variable and aboveground mass as the covariate for *Cakile edentula* seedlings grown in sterile water in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 86. N=95. Overall model significance is $P < 0.0256$.

Source	d.f.	Root allocation	
		F-ratio	P-value
Aboveground mass	1	13.32	0.0004
Family (F)	2	0.40	0.6746
Social Environment (S)	2	2.29	0.1077
F × S	4	0.72	0.5795

Table 3.1f: Specific root length from analysis of covariance of total root length as the dependent variable and root mass as the covariate for *Cakile edentula* seedlings grown in sterile water in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 86. N=95. Overall model significance is $P < 0.0025$.

Source	d.f.	Specific root length	
		F-ratio	P-value
Root mass	1	17.04	<0.0001
Family (F)	2	1.20	0.2939
Social Environment (S)	2	0.06	0.9435
F × S	4	2.24	0.0711

Figure 3.1a: Effect of social environment and family on number of laterals for *Cakile edentula* seedlings grown in sterile water in well-plates switched with either kin or stranger seedling for 14 days. The bars indicate ± 1 standard error.

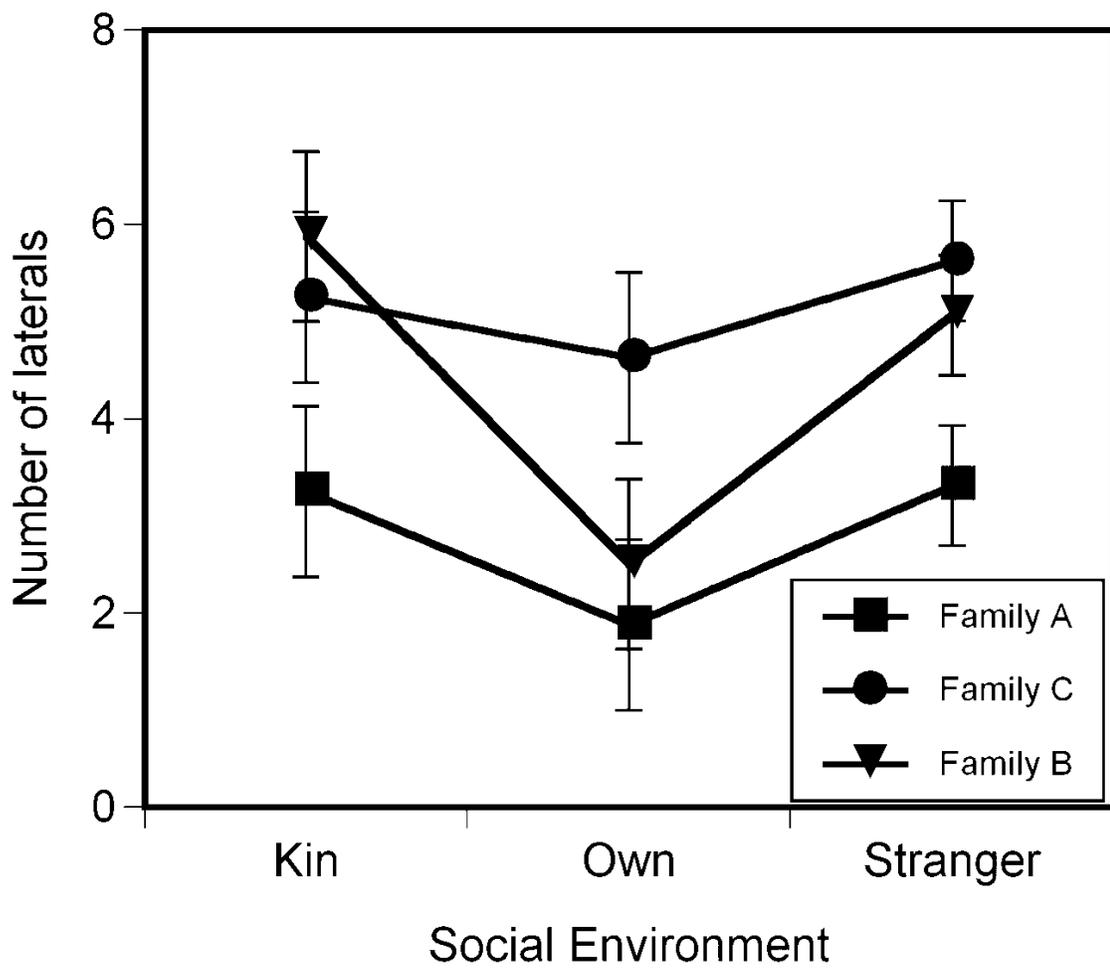
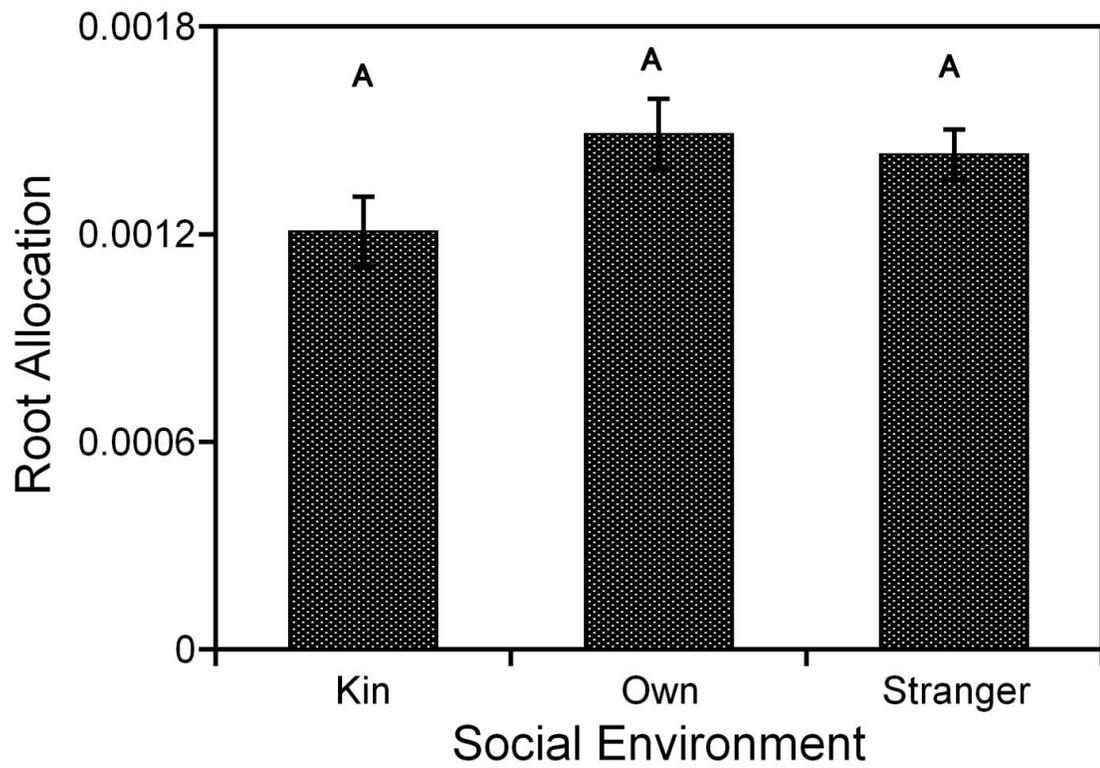


Figure 3.1b: Bar graph of root allocation (least square means of root biomass with aboveground biomass as the covariate) for *Cakile edentula* seedlings, grown in sterile water in well-plates switched with either kin or stranger seedling for 14 days, in response to social environment. The letters indicate statistically significant differences between the social environment treatments at the 0.05 level. The bars indicate ± 1 standard error..



3.2 Nutrient-Rich experiment

For this experiment, seedlings from four families were grown in liquid MS media (a nutrient-rich environment) for 14 days. There were no significant main effects of social environment and family on tap-root length ($p < 0.2410$), total lateral length ($p < 0.7115$), number of laterals ($p < 0.4005$), total root length ($p < 0.5773$), hypocotyl length ($p < 0.0927$), total mass ($p < 0.9891$), or aboveground mass ($p < 0.9834$) (Table 3.2a, 3.2b and 3.2c). The root mass ($p < 0.0042$) was affected by social environment but did not differ among families, with seedlings in stranger social environment having greater root mass than seedlings in kin or own social environment (Table 3.2c). Significant effects of social environment were observed for morphological and allocation traits (Table 3.2d, 3.2e, 3.2 f). Seedlings exposed to stranger exudates had decreased root elongation and increased root allocation compared to seedlings exposed to kin or own exudates (Figure 3.2a, 3.2b). Seedlings exposed to stranger exudates had significantly decreased specific root length compared to seedlings exposed to own exudates. Seedlings exposed to kin exudates did not differ in specific root length from seedlings exposed to stranger or own exudates (Figure 3.2c).

Table 3.2a: Analysis of variance for root architecture traits of *Cakile edentula* seedlings grown in liquid MS media in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 94. N = 105. Family is C, F, G or H; Social environment is kin, stranger or own exudates.

Source	d.f.	Tap-root length		Number of laterals		Total lateral length		Total root length	
		F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
Family (F)	2	1.88	0.1390	0.51	0.6781	0.28	0.8366	0.97	0.4122
Social Environment (S)	2	1.04	0.3560	2.03	0.1370	1.50	0.2289	1.54	0.2195
F × S	4	1.22	0.3018	1.00	0.4332	0.64	0.6974	0.58	0.7467

Table 3.2b: Analysis of variance for aboveground trait of *Cakile edentula* seedlings grown in sterile liquid MS media in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 87. N = 95. Family is C, F, G or H; Social environment is kin, stranger or own exudates. Overall model significance is $P < 0.0927$.

Source	d.f.	Hypocotyl length	
		F-ratio	P-value
Family (F)	2	3.01	0.0341
Social Environment (S)	2	0.47	0.6247
F × S	4	1.05	0.3999

Table 3.2c: Analysis of variance for dry biomass of *Cakile edentula* seedlings grown in liquid MS media in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 94. N =105. Family is C, F, G or H; Social environment is kin, stranger or own exudates.

Source	d.f.	Total mass		Root mass		Aboveground mass	
		F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
Family (F)	2	0.32	0.8115	0.47	0.7006	0.36	0.7851
Social Environment (S)	2	0.06	0.9457	12.74	<0.0001	0.01	0.9916
F × S	4	0.28	0.9431	0.66	0.6786	0.33	0.9170

Table 3.2d: Root elongation analysis from analysis of covariance of tap-root length as the dependent variable and root mass as the covariate for *Cakile edentula* seedlings grown in liquid MS media in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 92. N=104. Overall model significance is $P < 0.0089$.

Source	d.f.	Root elongation	
		F-ratio	P-value
Root mass	1	12.88	0.0005
Family (F)	2	2.28	0.0843
Social Environment (S)	3	3.58	0.0320
F × S	6	2.13	0.0570

Table 3.2e: Root allocation analysis from analysis of covariance of root mass as the dependent variable and aboveground mass as the covariate for *Cakile edentula* seedlings grown in liquid MS media in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 92. N=104. Overall model significance is $P < 0.0001$.

Source	d.f.	Root allocation	
		F-ratio	P-value
Aboveground mass	1	44.60	<0.0001
Family (F)	2	1.19	0.3183
Social Environment (S)	3	17.39	<0.0001
F × S	6	1.58	0.1631

Table 3.2f: Specific root length from analysis of covariance of total root length as the dependent variable and root mass as the covariate for *Cakile edentula* seedlings grown in liquid MS media in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 92. N=104. Overall model significance is $P < 0.0060$.

Source	d.f.	Specific root length	
		F-ratio	P-value
Root mass	1	18.58	<0.0001
Family (F)	2	1.34	0.2653
Social Environment (S)	3	4.26	0.0170
F × S	6	1.13	0.3535

Figure 3.2a: Bar graph of root elongation (least square means of tap-root length with root biomass as the covariate) for *Cakile edentula* seedlings, grown in liquid MS media in well-plates, switched with either kin or stranger seedling for 14 days, in response to social environment. The letters indicate statistically significant differences between the social environment treatments at the 0.05 level. The bars indicate ± 1 standard error.

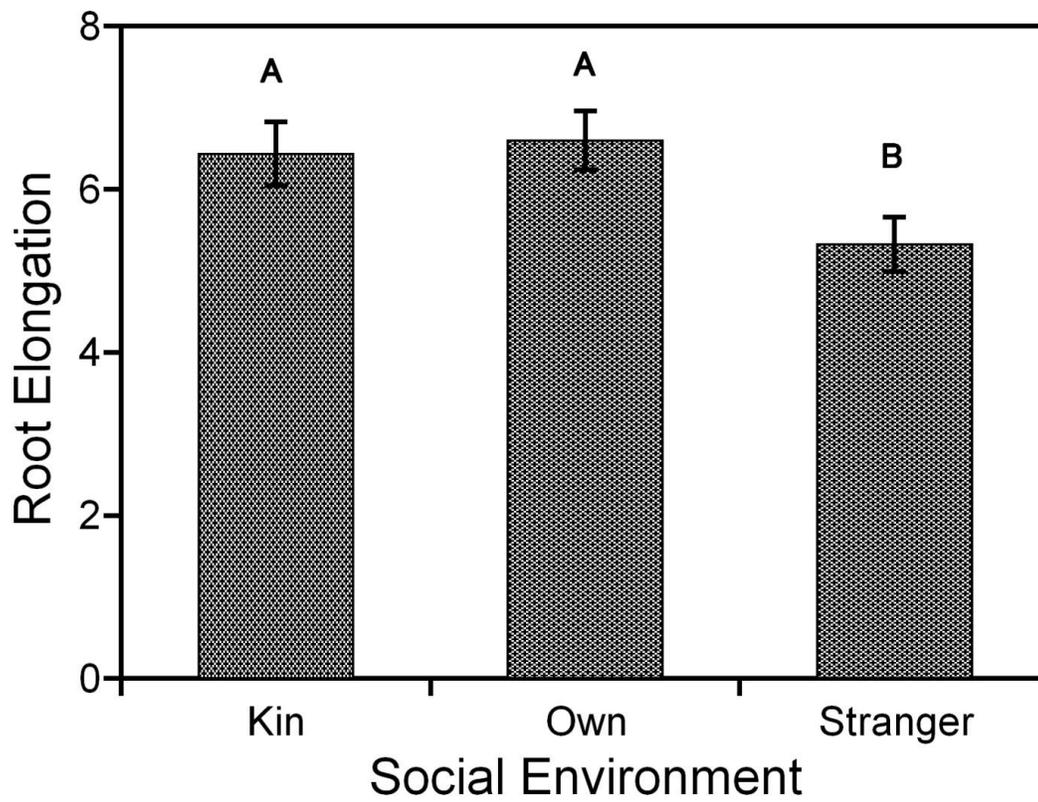


Figure 3.2b: Bar graph of root allocation (least square means of root mass with aboveground biomass as the covariate) for *Cakile edentula* seedlings, grown in liquid MS media in well-plates switched with either kin or stranger seedling for 14 days, in response to social environment. The letters indicate statistically significant differences between the social environment treatments at the 0.05 level. The bars indicate ± 1 standard error.

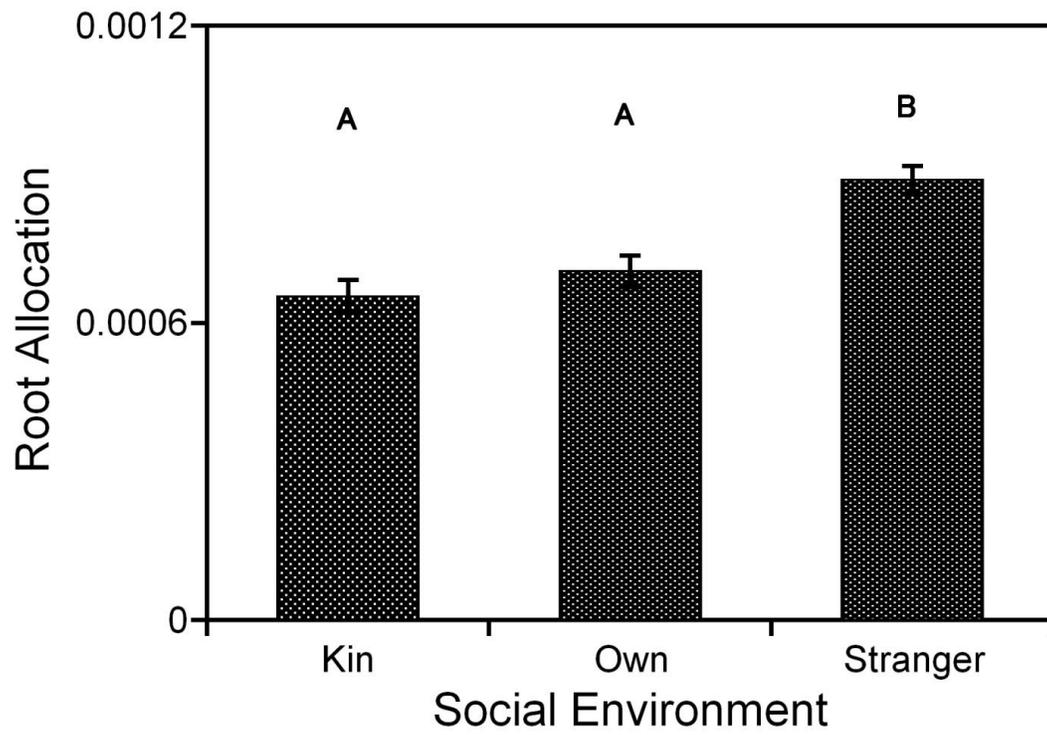
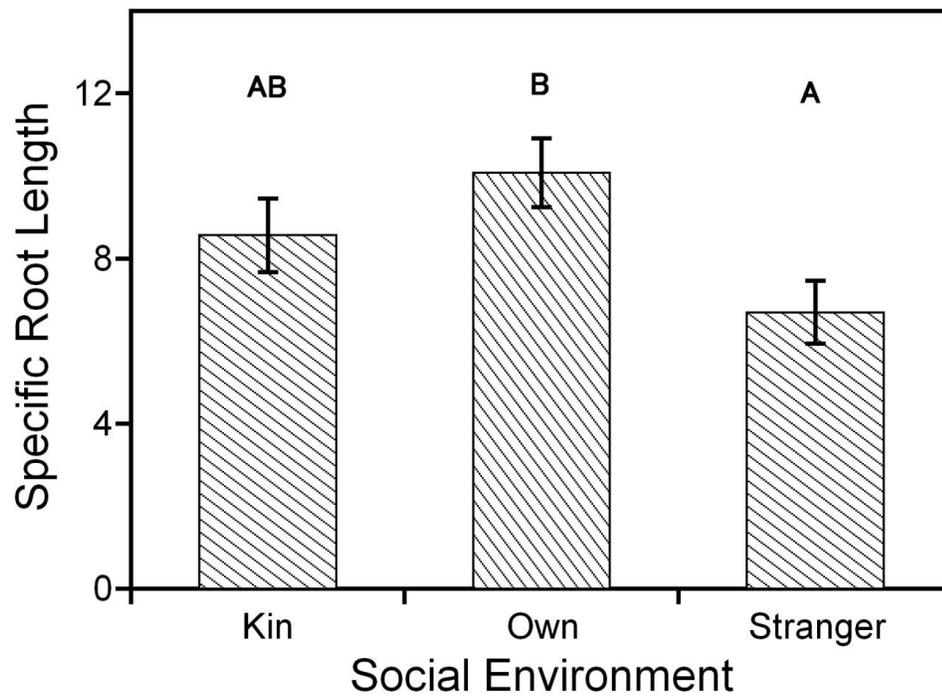


Figure 3.2c: Bar graph of specific root length (least square means of total root length with root biomass as the covariate) for *Cakile edentula* seedlings, grown in liquid MS media in well-plates switched with either kin or stranger seedling for 14 days, in response to social environment. The letters indicate statistically significant differences between the social environment treatments at the 0.05 level. The bars indicate ± 1 standard error.



3.3 Nutrient / no-nutrient combined experiment with four families

For this experiment, seedlings from four families were grown in either liquid MS media (nutrient rich environment) or sterile water (no- nutrient environment) and exposed to their own exudates or exudates from kin or stranger seedlings for 14 days. The nutrient environment had a significant effect on several aboveground and below ground traits. Tap-root ($p < 0.0034$), total biomass ($p < 0.0001$), hypocotyl length ($p < 0.0057$) and above-ground mass ($p < 0.0114$) were significantly lower and the number of laterals ($p < 0.0001$) were significantly greater for seedlings grown in no-nutrient environment than seedlings grown in nutrient-rich environment. The social environment had no effect on total lateral length, total root length, number of laterals, total biomass, tap root length, and aboveground mass traits. However, there were significant social environment \times nutrient environment interactions for tap-root length and hypocotyl length (Table 3.3a, 3.3b, 3.3c). When nutrients were available, seedlings exposed to their own exudates had longer tap root lengths than seedlings exposed to kin or stranger exudates. However, when no nutrients were available, there was no significant difference in tap root length between seedlings exposed to kin, stranger and own exudates (Figure 3.3a). Similarly, when nutrients were available, seedlings exposed to their own exudates had longer hypocotyl length than seedlings exposed to kin or stranger exudates. Seedlings grown in the no-nutrient environment did not display a significant difference in hypocotyl length when exposed to kin, stranger and own ($p < 0.0057$). The root biomass was affected by the social environment but not by nutrient environment, with lower root biomass in seedlings exposed to kin and own exudates than stranger exudates (Table 3.3a).

Root elongation and specific root length demonstrated significant environment \times nutrient environment interactions. When there were nutrients available, seedlings exposed to own exudates had more root elongation and specific root length than seedlings exposed to kin or stranger exudates. However, when nutrients were absent, there was no difference in root elongation and specific root length for seedlings exposed to kin, own or stranger exudates (Table 3.3d, 3.3f, Figure 3.3b, 3.3c).

There were significant main effects of social environment and nutrient environment on root allocation traits. Seedlings grown in nutrients allocated less to roots than seedlings grown in no nutrient environment (Figure 3.3e). There was significantly greater root allocation in seedlings exposed to stranger exudates than to kin or own exudates (Figure 3.3d). However, this effect of social environment did not change with nutrient availability, as indicated by a non-significant social environment \times nutrient environment interaction (Table 3.3e).

Table 3.3a: Analysis of variance for root architecture traits of *Cakile edentula* seedlings grown in liquid MS media or sterile water in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 228. N = 251. Family is C, D, B, E; Social environment is kin, stranger or own exudates; nutrient environment is nutrient and no nutrient.

Source	d.f.	Tap-root length		ln(Total lateral length)		Number of lateral		Total root length	
		F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
Family (F)	3	0.11	0.9524	3.17	0.0255	4.82	0.0028	0.94	0.4211
Social Environment (S)	2	2.33	0.1000	0.70	0.4967	1.49	0.2270	2.75	0.0661
S × F	6	1.21	0.3036	0.93	0.4730	0.84	0.5382	0.47	0.8322
Nutrients (N)	1	4.06	0.0451	0.00	0.9718	17.48	<0.0001	1.84	0.1764
F × N	3	4.04	0.0080	1.54	0.2057	5.89	0.0007	2.52	0.0584
S × N	2	5.46	0.0048	1.72	0.1812	1.91	0.1501	5.28	0.0057
S × F × N	6	1.78	0.1042	1.28	0.2658	2.17	0.0466	2.03	0.0630

Table 3.3b: Analysis of variance for Hypocotyl length of *Cakile edentula* seedlings grown in sterile liquid MS media or sterile water in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 228. N = 251. Family is C, D, B, E; Social environment is kin, stranger or own exudates; Nutrient environment is nutrient and no nutrient.

Source	d.f.	Hypocotyl length	
		F-ratio	P-value
Family (F)	3	2.75	0.0438
Social Environment (S)	2	0.07	0.9345
S × F	6	0.67	0.6748
Nutrients (N)	1	13.69	0.0003
F × N	3	2.18	0.0916
S × N	2	3.15	0.0448
S × F × N	6	0.50	0.8072

Table 3.3c: Analysis of variance for dry biomass of *Cakile edentula* seedlings grown in liquid MS media or sterile water in well-plates switched with either kin or stranger seedling for 14 days. Error d.f. is 228. N = 251. Family is C, D, B, E; Social environment is kin, stranger or own exudates; Nutrient environment is nutrient and no nutrient.

Source	d.f.	Total mass		Root mass		Aboveground mass	
		F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
Family (F)	3	4.62	0.0037	4.71	0.0033	4.36	0.0052
Social Environment (S)	2	1.07	0.3445	5.36	0.0053	1.44	0.2387
S × F	6	0.45	0.8475	0.75	0.6107	0.49	0.8123
Nutrients (N)	1	136.96	<0.0001	2.06	0.1528	146.21	<0.0001
F × N	3	4.40	0.0049	3.85	0.0103	4.43	0.0047
S × N	2	2.82	0.0617	2.21	0.1119	2.79	0.0634
S × F × N	6	0.69	0.6546	1.39	0.2195	0.69	0.6582

Table 3.3d: Root elongation analysis from analysis of covariance of tap-root length as the dependent variable and root mass as the covariate for *Cakile edentula* seedlings grown in liquid MS media or sterile water in well-plates switched with either kin or stranger seedling for 14 days. Family is C, D, B, E; Social environment is kin, stranger or own exudates; Nutrient environment is nutrient and no nutrient. Error d.f. is 226. N=250. Overall model significance is $P < 0.0001$.

Source	d.f.	Root elongation	
		F-ratio	P-value
Root mass	1	50.06	<0.0001
Family (F)	2	6.07	0.2128
Social Environment (S)	1	7.94	0.0027
S × F	2	4.06	0.0682
Nutrients Environment (N)	3	1.51	0.0053
F × N	6	1.99	0.0011
S × N	3	5.53	0.0186
S × F × N	6	1.42	0.2082

Table 3.3e: Root allocation analysis from analysis of covariance of root mass as the dependent variable and aboveground mass as the covariate for *Cakile edentula* seedlings grown in liquid MS media or sterile water in well-plates switched with either kin or stranger seedling for 14 days. Family is C, D, B, E; Social environment is kin, stranger or own exudates; Nutrient environment is nutrient and no nutrient. Error d.f. is 226. N=250. Overall model significance is $P < 0.0001$.

Source	d.f.	Root allocation	
		F-ratio	P-value
Aboveground mass	1	40.45	<0.0001
Family (F)	2	9.10	0.0406
Social Environment (S)	1	27.05	0.0002
S × F	2	1.60	0.3381
Nutrients Environment (N)	3	2.80	<0.0001
F × N	6	1.14	0.0045
S × N	3	4.47	0.2039
S × F × N	6	1.45	0.1979

Table 3.3f: Specific root length from analysis of covariance of total root length as the dependent variable and root mass as the covariate for *Cakile edentula* seedlings grown in liquid MS media in well-plates switched with either kin or stranger seedling for 14 days. Family is C, D, B, E; Social environment is kin, stranger or own exudates; Nutrient environment is nutrient and no nutrient. Error d.f. is 226. N=250. Overall model significance is $P < 0.0001$.

Source	d.f.	Specific root length	
		F-ratio	P-value
Root mass	1	79.95	<0.0001
Family (F)	2	9.28	0.9457
Social Environment (S)	1	5.57	0.0001
S × F	2	4.41	0.3991
Nutrients (N)	3	0.12	0.0191
F × N	6	1.04	0.0033
S × N	3	4.70	0.0132
S × F × N	6	1.35	0.2349

Figure 3.3a: Effect of social environment and nutrient environment on tap-root length for *Cakile edentula* seedlings grown either sterile water or liquid MS media in well-plates and switched with either kin or stranger seedling for 14 days. The bars indicate ± 1 standard error.

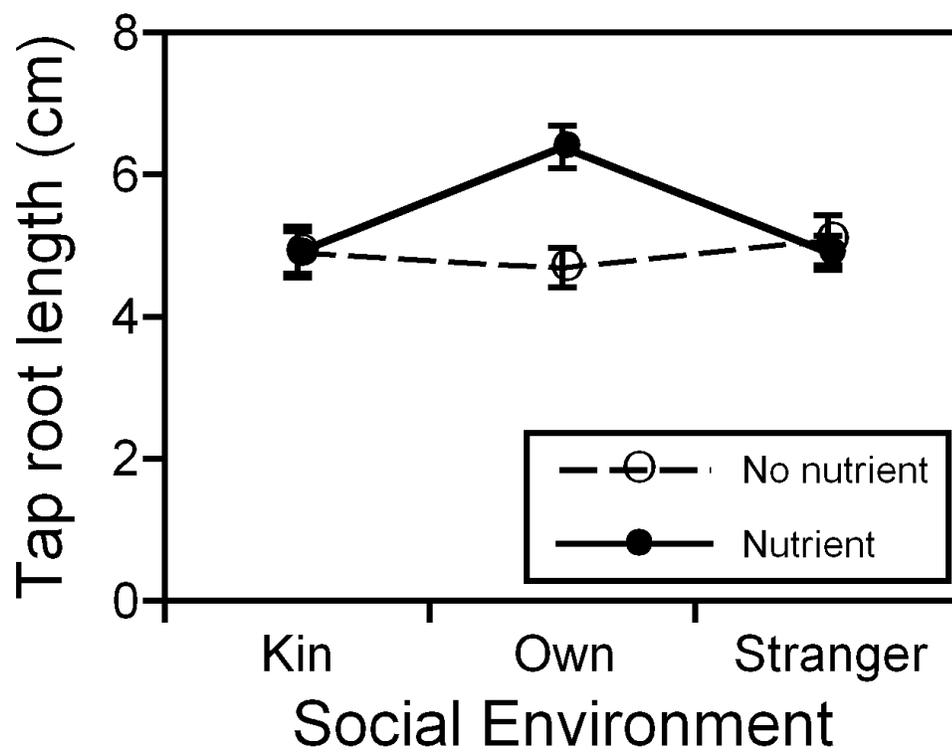


Figure 3.3b: Effect of social environment and nutrient environment on root elongation (least square means of tap-root length with root biomass as the covariate) for *Cakile edentula* seedlings grown in either sterile water or liquid MS media in well-plates and switched with either kin or stranger seedling for 14 days. The bars indicate ± 1 standard error.

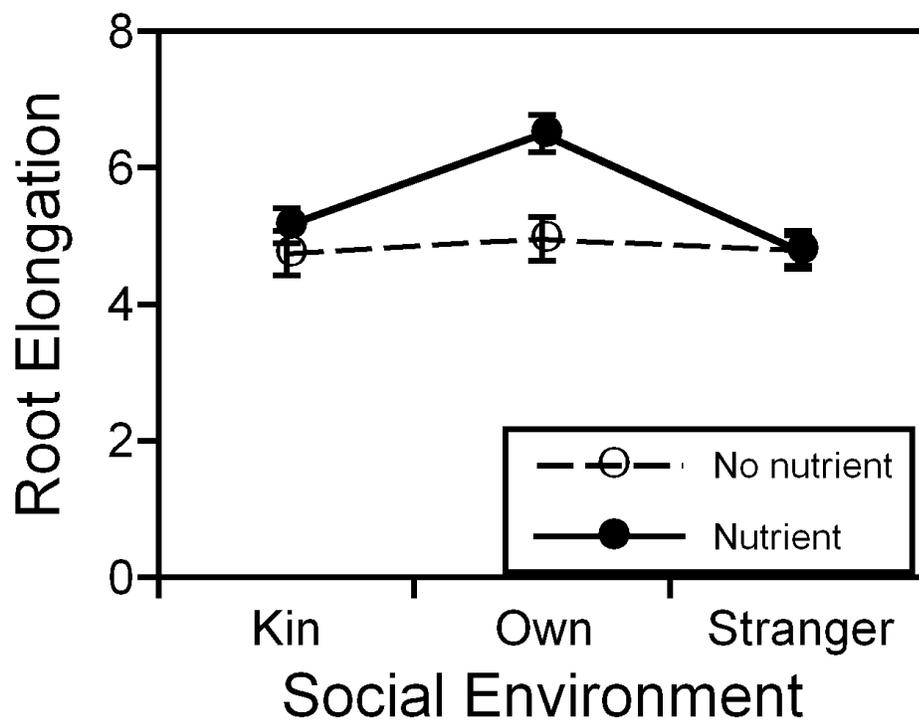


Figure 3.3c: Effect of social environment and nutrient environment on specific root length (least square means of total root length with root biomass as the covariate) for *Cakile edentula* seedlings grown in either sterile water or liquid MS media in well-plates and switched with either kin or stranger seedling for 14 days. The bars indicate ± 1 standard error.

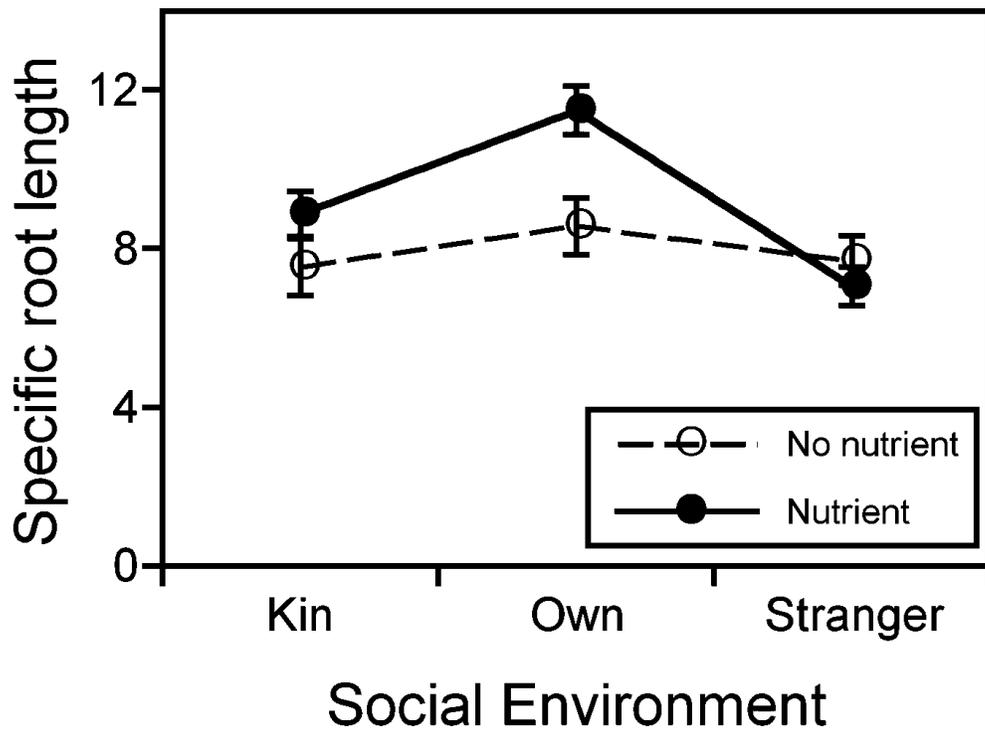


Figure 3.3d: Bar graph showing the effect of social environment on root allocation (least square means of root mass with aboveground biomass as the covariate) for *Cakile edentula* seedlings, grown in either sterile water or liquid MS media in well-plates switched with either kin or stranger seedling for 14 days, in response to social environment. The letters indicate statistically significant differences between the social environment treatments at the 0.05 level. The bars indicate ± 1 standard error.

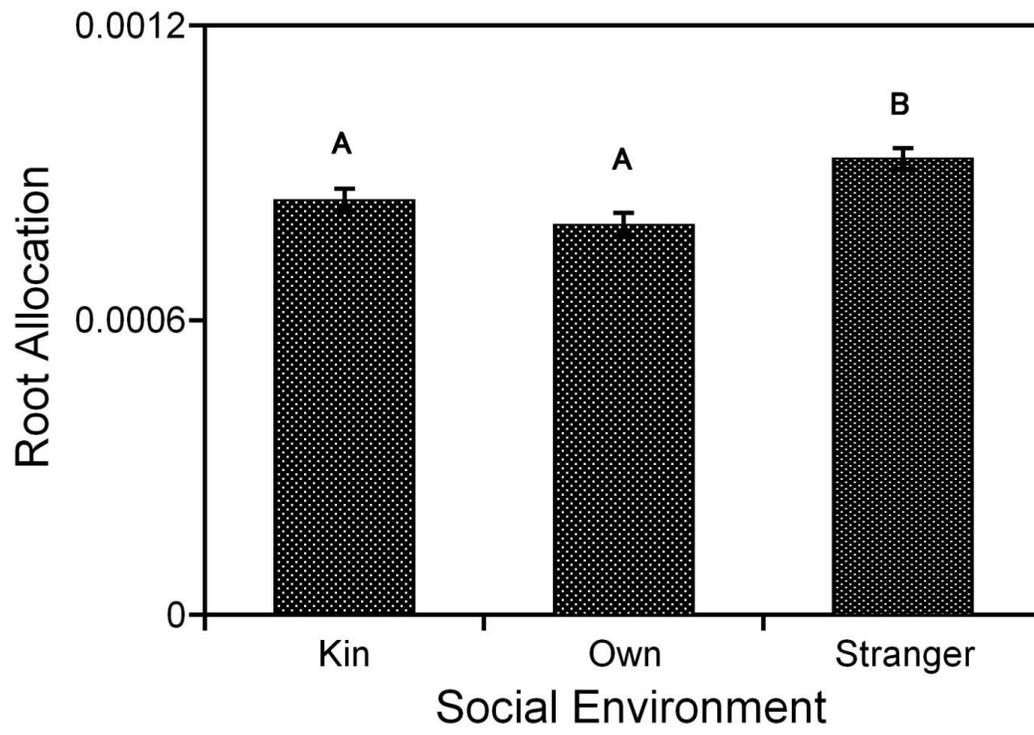
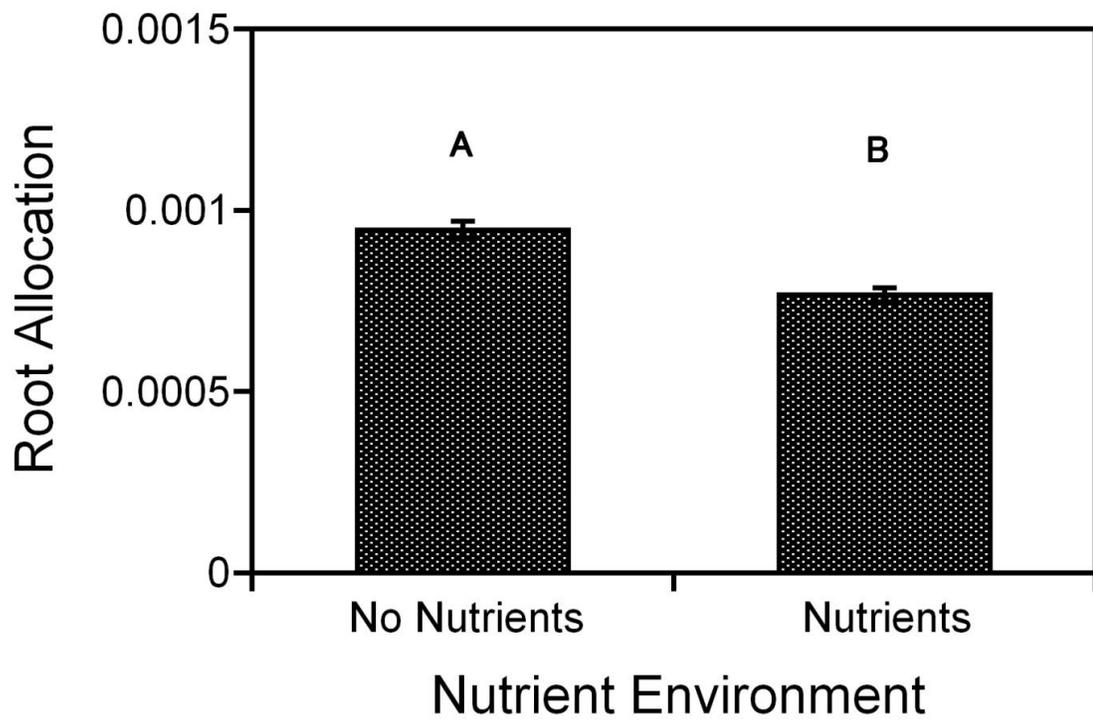


Figure 3.3e: Bar graph showing the effect of nutrient environment on root allocation (least square means of root mass with aboveground biomass as the covariate) for *Cakile edentula* seedlings, grown in either sterile water or liquid MS media in well-plates switched with either kin or stranger seedling for 14 days, in response to social environment. The letters indicate statistically significant differences between the social environment treatments at the 0.05 level. The bars indicate ± 1 standard error.



3.4 Nutrient / no-nutrient combined experiment with two families

For this experiment, seedlings from two families were grown in either liquid MS media (nutrient rich environment) or sterile water (no- nutrient environment) for 7 days to increase the sample size. Tap-root length ($p < 0.0001$), total biomass ($p < 0.0001$), hypocotyl length ($p < 0.0001$), above-ground mass ($p < 0.0001$) were significantly greater in seedlings grown in no nutrient environment than seedlings grown in nutrient environment. But the social environment had no significant effect on these traits. There were significant main effects of social environment and nutrient environment on total lateral length ($p < 0.0001$), number of laterals ($p < 0.0002$), total root length ($p < 0.0001$). Seedlings exposed to kin or stranger exudates had lower total lateral length and total root length than seedlings to own exudates. Similarly, seedlings had lower number of laterals when exposed to kin exudates but not with the stranger or own exudates. Seedlings grown in nutrient environment were bigger than seedlings grown in no nutrient environment. There was no significant social environment \times nutrient availability interaction in any these measured traits (Table 3.4a, 3.4b, 3.4c). The root biomass was affected by the social environment and nutrient environment, with highest root biomass in seedlings exposed to stranger exudates, intermediate root biomass for seedlings exposed to own exudates and lowest root biomass in seedlings exposed to kin exudates and greater root biomass in the nutrient-rich environment than in nutrient-deprived environment (Table 3.4a).

There was a significant social environment \times nutrient availability interaction on root elongation and specific root length trait with social environment affecting total root elongation and specific root length when nutrients are present but not affecting these traits

when no nutrients are present. When nutrients are available, seedlings grown in kin or own exudates had significantly more root elongation (longer tap root relative to total root biomass) and specific root length (longer roots relative to total root biomass) compared to seedlings grown in stranger exudates. When no nutrients are available, the root elongation and specific root length did not differ between the kin, own and strangers (Table 3.4d, Figure 3.4).

Similarly, there was a significant social environment \times nutrient availability interaction on root allocation trait with social environment affecting root allocation when nutrients are present but not affecting root allocation when no nutrients are present. When nutrients were available, seedlings grown in stranger exudates had allocated significantly more to root relative to aboveground mass compared to seedlings exposed to kin or own exudates. However, root allocation did not differ between kin, own and stranger when no nutrients are available (Table 3.4e, Figure 3.4b)

Table 3.4a: Analysis of variance for root architecture traits of *Cakile edentula* seedlings grown in liquid MS media or sterile water in well-plates switched with either kin or stranger seedling for 7 days. Error d.f. is 166. N = 177. Family is C or E; Social environment is kin, stranger or own exudates; nutrient environment is nutrient and no nutrient.

Source	d.f.	Tap-root length		Total lateral length		Number of lateral		Total root length	
		F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
Family (F)	1	10.25	0.0016	9.40	0.0025	7.28	0.0077	12.51	0.0005
Social Environment (S)	2	2.26	0.1072	4.70	0.0104	4.81	0.0093	4.87	0.0088
S × F	2	0.17	0.8441	0.62	0.5401	0.55	0.5772	0.58	0.5617
Nutrients (N)	1	13.08	0.0004	8.19	0.0047	11.60	0.0008	12.40	0.0006
F × N	1	9.62	0.0023	4.50	0.0353	3.63	0.0584	7.60	0.0065
S × N	2	1.86	0.1586	1.65	0.1959	0.65	0.5249	2.21	0.1125
S × F × N	2	0.04	0.9653	1.38	0.2551	0.55	0.5757	0.98	0.3765

Table 3.4b: Analysis of variance for aboveground trait of *Cakile edentula* seedlings grown in sterile liquid MS media or sterile water in well-plates switched with either kin or stranger seedling for 7 days. Error d.f. is 166. N = 177. Family is C or E; Social environment is kin, stranger or own exudates; nutrient environment is nutrient and no nutrient.

Source	d.f.	Hypocotyl length	
		F-ratio	P-value
Family (F)	1	65.42	<0.0001
Social Environment (S)	2	0.91	0.4043
S × F	2	0.37	0.6916
Nutrients Environment (N)	1	30.38	<0.0001
F × N	1	8.86	0.0033
S × N	2	0.19	0.8234
S × F × N	2	1.03	0.3610

Table 3.4c: Analysis of variance for dry biomass of *Cakile edentula* seedlings grown in liquid MS media or sterile water in well-plates switched with either kin or stranger seedling for 7 days. Error d.f. is 166. N = 177. Family is C or E; Social environment is kin, stranger or own exudates; nutrient environment is nutrient and no nutrient.

Source	d.f.	Total mass		Root mass		Aboveground mass	
		F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
Family (F)	1	155.78	<0.0001	20.45	<0.0001	159.18	<0.0001
Social Environment (S)	2	2.47	0.0878	5.13	0.0069	2.17	0.1169
S × F	2	0.07	0.9338	0.40	0.6722	0.10	0.9037
Nutrients Environment (N)	1	16.87	<0.0001	20.66	<0.0001	15.01	0.0002
F × N	1	3.67	0.0572	5.21	0.0237	3.21	0.0750
S × N	2	0.09	0.9135	6.03	0.0030	0.01	0.9916
S × F × N	2	1.74	0.1795	1.31	0.2728	1.88	0.1554

Table 3.4d: Root elongation analysis from analysis of covariance of tap-root length as the dependent variable and root mass as the covariate for *Cakile edentula* seedlings grown in liquid MS media or sterile water in well-plates switched with either kin or stranger seedling for 7 days. Error d.f. is 165. N = 177; Family is C or E; Social environment is kin, stranger or own exudates; Nutrient environment is nutrient and no nutrient. Overall model significance is $P < 0.0001$.

Source	d.f.	Root elongation	
		F-ratio	P-value
Root mass	1	83.47	<0.0001
Family (F)	1	0.45	0.5043
Social Environment (S)	2	6.01	0.0030
S × F	2	0.75	0.4733
Nutrients (N)	1	1.28	0.2586
F × N	1	4.59	0.0337
S × N	2	8.00	0.0005
S × F × N	2	1.06	0.3475

Table 3.4e: Root allocation analysis from analysis of covariance of root mass as the dependent variable and aboveground mass as the covariate for *Cakile edentula* seedlings grown in liquid MS media or sterile water in well-plates switched with either kin or stranger seedling for 7 days. Error d.f. is 166. N=177; Family is C or E; Social environment is kin, stranger or own exudates; Nutrient environment is nutrient and no nutrient. Overall model significance is $P < 0.0001$.

Source	d.f.	Root allocation	
		F-ratio	P-value
Aboveground mass	1	41.27	<0.0001
Family (F)	1	0.80	0.3733
Social Environment (S)	2	3.97	0.0206
S × F	2	0.70	0.4968
Nutrients (N)	1	9.01	0.0031
F × N	1	2.068	0.1036
S × N	2	7.24	0.0010
S × F × N	2	2.10	0.1252

Table 3.4f: Specific root length from analysis of covariance of total root length as the dependent variable and root mass as the covariate for *Cakile edentula* seedlings grown in liquid MS media in well-plates switched with either kin or stranger seedling for 7 days. Error d.f. is 165. N=177. Family is C or E; Social environment is kin, stranger or own exudates; nutrient environment is nutrient and no nutrient. Overall model significance is $P < 0.0001$.

Source	d.f.	Specific root length	
		F-ratio	P-value
Root mass	1	61.74	<0.0001
Family (F)	1	1.68	0.1962
Social Environment (S)	2	6.74	0.0015
S × F	2	1.48	0.2307
Nutrients (N)	1	1.60	0.2071
F × N	1	3.24	0.0735
S × N	2	7.04	0.0012
S × F × N	2	3.39	0.0360

Figure 3.4a: Effect of social environment and nutrient environment on root elongation (least square means of tap-root length with root biomass as the covariate) for *Cakile edentula* seedlings grown in either sterile water or liquid MS media in well-plates and switched with either kin or stranger seedling for 7 days. The bars indicate ± 1 standard error.

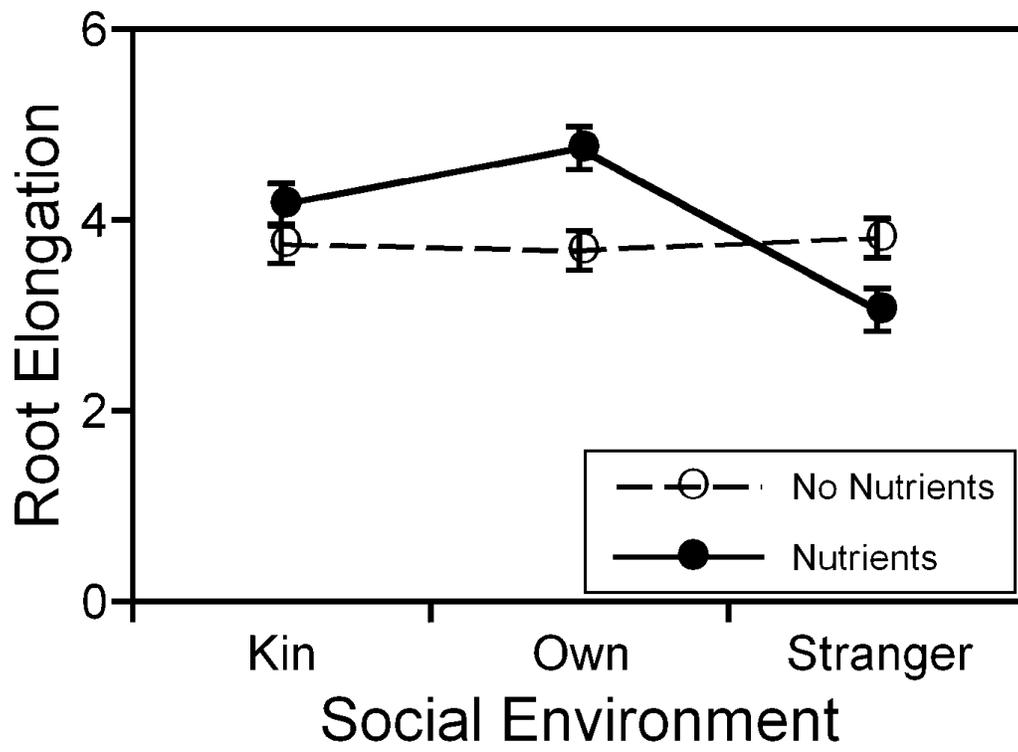


Figure 3.4b: Effect of social environment and nutrient environment on specific root length (least square means of total root length with root biomass as the covariate) for *Cakile edentula* seedlings grown in either sterile water or liquid MS media in well-plates and switched with either kin or stranger seedling for 7 days. The bars indicate ± 1 standard error.

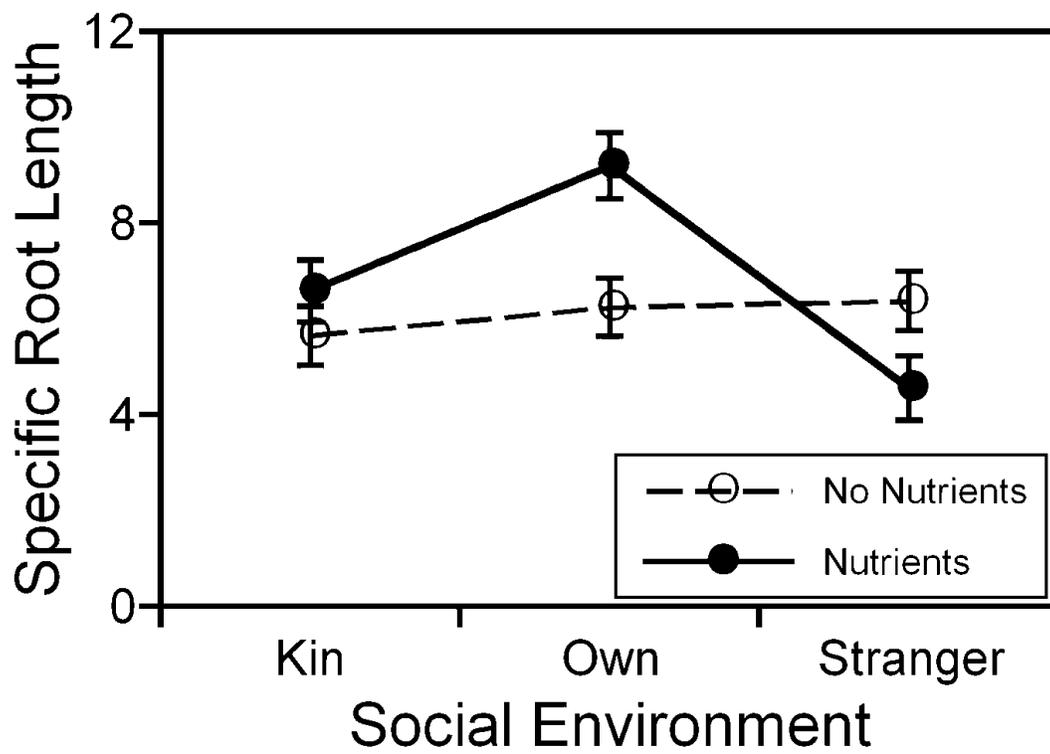
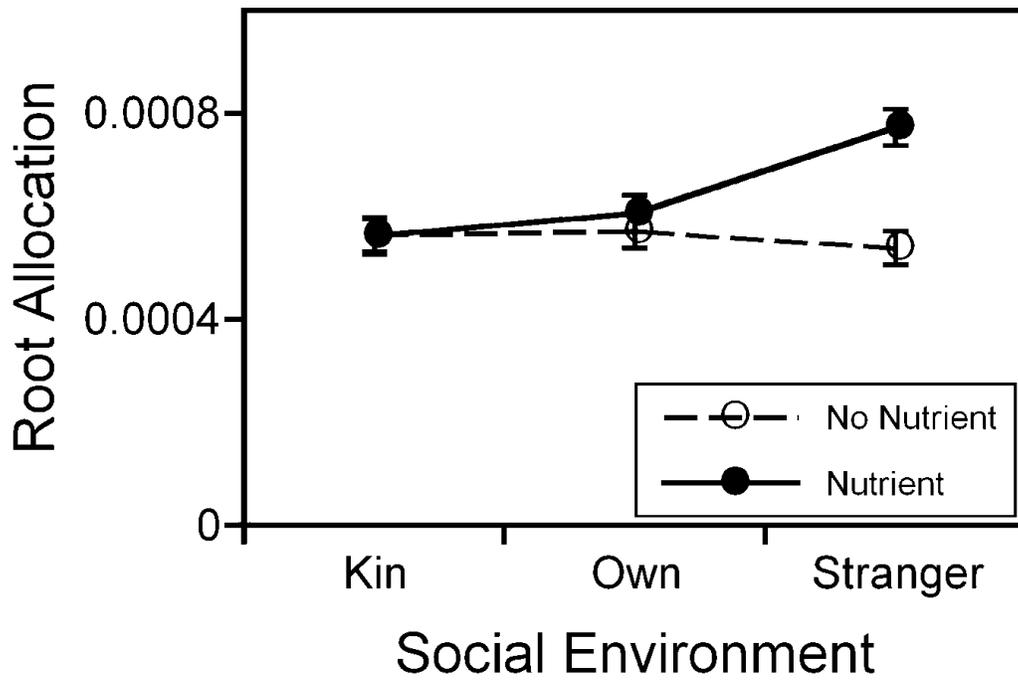


Figure 3.4c: Effect of social environment and nutrient environment on root allocation (least square means of total root mass with aboveground biomass as the covariate) for *Cakile edentula* seedlings grown in either sterile water or liquid MS media in well-plates and switched with either kin or stranger seedling for 7 days. The bars indicate ± 1 standard error.



CHAPTER 4: CONCLUSION

I carried out four experiments designed to determine whether in *C. edentula* seedlings, the response to social environment is mediated by exudates, and how nutrient availability affects this response. Seedlings in these experiments responded to conditioned media, based on the identity of the seedling which had been grown in the media without root contact, providing strong support for the hypothesis that root exudates is the basis of kin recognition and that root contact is not necessary to signal neighbour identity in *C. edentula*. These results are consistent with the mechanism of kin recognition observed by Biedrzycki et al (2010) in *A. thaliana*. The kin recognition response observed in the nutrient-rich environment, with higher root allocation in seedlings exposed to stranger exudates than kin seedlings, was consistent with those previously observed in 8 week old juvenile plants (Dudley and File 2007) and 6 and 3 week old young seedlings (Bhatt, Khandelwal et al. 2011). However, seedlings did not respond to neighbour relatedness when grown in nutrient- deprived environment. These results indicate that kin recognition response through root allocation observed in *C. edentula* is dependent on nutrient availability.

Across the four experiments, a consistent trend was that seedlings grown in the nutrient-deprived environment, fewer below-ground traits responded to social environment, while in the nutrient-rich environment, several below-ground traits responded to social environment. In experiment 1 (no nutrients), experiment 3 (no nutrient treatment), and experiment 4 (no nutrient treatment), root allocation did not respond to exudate source. In experiment 2 (nutrients supplied), experiment 3 (nutrient

treatment), and experiment 4 (nutrient treatment), we found a trend that seedlings exposed to stranger exudates increase root allocation. Because experiment 1 and 2 only had one nutrient environment in each, we could not test for a social environment \times nutrient interaction. In experiment 3, we used a factorial design, but the social environment \times nutrient interaction was not significant. We observed a strong family \times nutrient interaction which may have reduced our power to test for the social environment \times nutrient interaction. In experiment 4, two families that responded similarly to nutrients were used. In experiment 4, there was a social environment \times nutrient interaction with root allocation in seedlings exposed to stranger exudates significantly higher than seedlings exposed to own and kin exudates. This suggests that seedlings exposed to stranger exudates are investing more resources to belowground traits, which would allow them to exhibit a competitive response towards possible root neighbours only when the basic nutrient needs are met. However, the observed responses to social and nutrient environment are more complex than previously realized, affecting multiple components of plant architecture.

The foraging strategies of an organism play an essential role in resource acquisition and are broadly dependent on organism's life history traits and its natural environment (Cahill and McNickle 2011). For a seedling, the phenotypic plasticity in root morphology and allocation traits may be a part of its foraging strategies. Seedlings exposed to the stranger exudates have increased root allocation and reduced root elongation, producing shorter thicker roots. This root morphology is similar to developing roots that act as storage organs (Fitter, Strickland et al. 1991). The literature suggests that

thicker roots tend to have lower root absorption potential and higher storage capacity as they have higher root density (Paula, 2011). However, there is a trade-off between resource storage and resource acquisition in roots. Since, *C. edentula* seedlings were grown in a nutrient-rich liquid media, there were no apparent limitations in water and resource acquisition. Consequently, we speculate that the root morphology observed in the seedlings exposed to the stranger social environment may be a strategy to conserve resources for future competition.

When grown in a nutrient- rich environment, seedlings grown in own exudates had similar root allocation as seedlings exposed to kin exudates; however, seedlings grown in own exudates had increased root elongation compared to seedlings exposed to kin exudates, thus producing longer thinner roots. Producing longer thinner roots would allow the plant to proliferate and forage below-ground resources found further away using the same carbon investment. Previous root architecture studies suggest that root system development depends on the presence or absence of a root neighbour (Fang, Clark et al. 2013) and resource distribution (Hodge 2004). Cahill et al. (2010) showed that solitarily grown *Abutilon theophrasti* plants adopt a broad foraging strategy, independent of resource distribution, while non-solitarily grown plants adopt a more restricted foraging strategy, which is dependent on resource distribution. Further, Nord et al. (2011) showed that in presence of a root neighbour, common bean plants alter their vertical and horizontal root distribution by placing fewer roots in space that is occupied by neighbour roots. Hence, the observed root morphology in seedlings exposed to their own exudates may be a strategy adapted to be a broad forager.

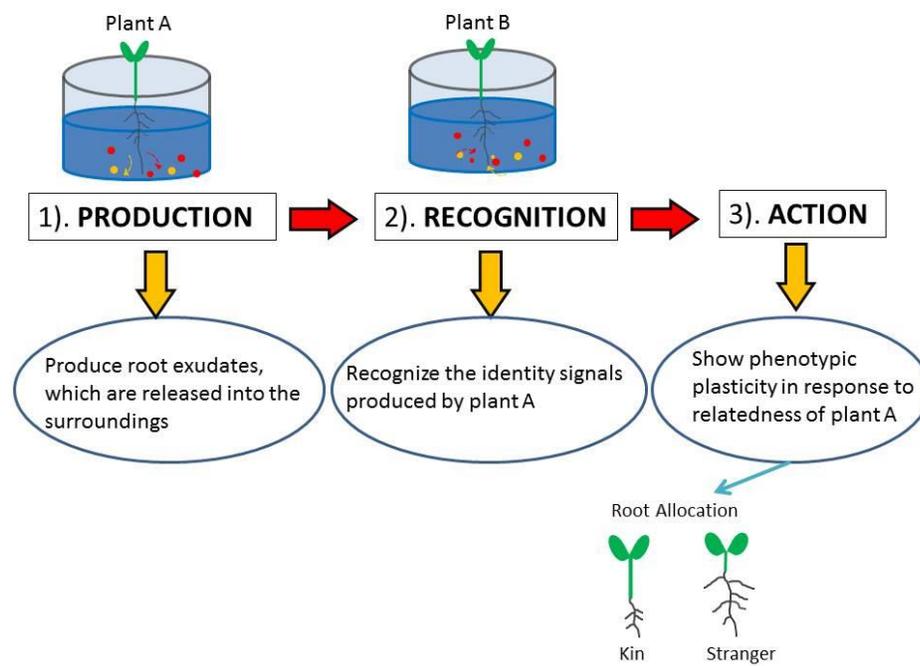
C. edentula showed different trends of self/non-self recognition response and kin recognition response in lateral morphology. However, these results were only present in one of the four experiments and may have been an anomaly. In experiment 4, *C. edentula* seedlings produced a decreased number of lateral roots when exposed to kin exudates than to own or stranger exudate in both the nutrient-rich and the nutrient-deprived environments. Lower number of laterals, a putative competitive trait, in seedlings exposed to kin would suggest a kin recognition response, by less investment in a belowground competitive trait. Additionally, the results from experiment 1 demonstrate a self /non-self- response in the number of laterals in *C. edentula*. In experiment 1, *C. edentula* seedlings exposed to kin or stranger exudates had significantly more number of laterals than seedlings exposed to their own exudates. This root morphology pattern indicates that lateral roots respond to self /non-self recognition in *C. edentula*. Similarly, the results from experiment 4 show a self /non- self- response in total lateral length trait in *C. edentula*. In experiment 4, *C. edentula* seedlings exposed to kin or stranger exudates had significantly less lateral root length than seedlings exposed to its own exudates. Patterns for self /non- self- response from both experiments contrast with self/non-self pattern in *A. thaliana* (Biedrzycki et al 2010), which demonstrated that self/ non-self recognition response in tap root length.

While these results demonstrate that root exudates provide identity signals and are the basis of kin recognition in *C. edentula*, the exact mechanism of the kin recognition signal is still unknown. We suggest three possibilities for the lack of the kin recognition response in seedlings that were grown in the nutrient-deprived environment. Firstly, it is

possible that when seedlings are deprived of nutrients, they do not produce the identity signals that would be recognized by the neighbor (Badri, Weir et al. 2009). The second possibility is that the identity signals are produced and released into the soil but are not recognized by the neighboring individual. Finally, the third possibility is that the identity signals are produced and released into the soil, recognized but ignored by the neighboring roots, producing no phenotypic change (Figure 4.1). Plants make complex decisions, throughout their lifetime, where a response to one cue depends on another one. Another example of complex decision making in plants comes from a study done on *Abutilon theophrasti* by Cahill et al. (2010). The study showed that root placement was determined by a neighbour's presence and resource distribution. These complex decision rules can be explored further with a future experiment analyzing the root exudate profile for seedlings grown in a nutrient-rich and a nutrient-deprived environment.

I was able to successfully develop a methodology to grow *C. edentula* seedlings sterilely. However, a future study using rhizotrons would provide a more in-depth understanding of the root architectural responses to social and nutrient environment in *C. edentula*. The complex belowground responses may vary if the seedlings were grown in soil or sand where they would have to forage for nutrient patches instead of them being readily available, when grown hydroponically in liquid MS Media. Hence, a root-box that allows us to non-destructively observe and quantify the growth of the root system ((Neufeld, Durall et al. 1989) would be ideal for further studying social by nutrient interactions in *C. edentula*.

Figure 4.1: Summary of the hypothesized complex decisions required in producing and recognizing identity signals. The lack of kin recognition response in root allocation in seedlings grown in the nutrient-deprived environment could be a result of an error in any of these three steps.



REFERENCES

- Badri, D. V., T. L. Weir, D. van der Lelie and J. M. Vivanco (2009). "Rhizosphere chemical dialogues: plant-microbe interactions." Current Opinion in Biotechnology **20**(6): 642-650.
- Bais, H. P., V. M. Loyola-Vargas, H. E. Flores and J. M. Vivanco (2001). "Root-Specific Metabolism: The Biology and Biochemistry of Underground Organs." In Vitro Cellular & Developmental Biology. Plant **37**(6): 730-741.
- Bais, H. P., S.-W. Park, T. L. Weir, R. M. Callaway and J. M. Vivanco (2004). "How plants communicate using the underground information superhighway." Trends in Plant Science **9**: 26-32.
- Bais, H. P., T. L. Weir, L. G. Perry, S. Gilroy and J. M. Vivanco (2006). "The role of root exudates in rhizosphere interactions with plants and other organisms." Annual Review of Plant Biology **57**: 233-266.
- Ballare, C., A. Scopel and R. Sanchez (1990). "Far-red radiation reflected from adjacent leaves: an early signal of competition in plant canopies." Science **247**: 329-333.
- Banks, S. C., D. B. Lindenmayer, L. McBurney, D. Blair, E. J. Knight and M. D. J. Blyton (2011). "Kin selection in den sharing develops under limited availability of tree hollows for a forest marsupial." Proceedings of the Royal Society B-Biological Sciences **278**(1719): 2768-2776.
- Barber, S. A. and M. Silberbush (1984). Chapter 4: Plant Root Morphology and Nutrient Uptake. Roots, Nutrient and Water Influx, and Plant Growth: 65-87.
- Bhatt, M. V., A. Khandelwal and S. A. Dudley (2010). "Kin recognition, not competitive interactions, predicts root allocation in young *Cakile edentula* seedling pairs." New Phytologist.
- Biedrzycki, M., T. Jilany, S. Dudley and H. Bais (2010). "Root exudates mediate kin recognition in plants." Communicative and Integrative Biology **3**(1): 1-8.
- Callaway, R. M. (2002). "The detection of neighbors by plants." Trends in Ecology & Evolution **17**(3): 104-105.
- Casper, B. B. and R. B. Jackson (1997). "Plant competition underground." Annual Review of Ecological Systematics **28**: 545-570.

- Cornelissen, J. H. C., S. Lavorel, E. Garnier, S. Diaz, N. Buchmann, D. E. Gurvich, P. B. Reich, H. ter Steege, H. D. Morgan, M. G. A. van der Heijden, J. G. Pausas and H. Poorter (2003). "A handbook of protocols for standardised and easy measurement of plant functional traits worldwide." Australian Journal of Botany **51**(4): 335-380.
- Cahill, J. F. and G. G. McNickle (2011). "The Behavioral Ecology of Nutrient Foraging by Plants." Annual Review of Ecology, Evolution, and Systematics, Vol **42**: 289-311
- de Kroon, H. and L. Mommer (2006). "Root foraging theory put to the test." Trends in Ecology & Evolution **21**(3): 113-116.
- Donohue, K. (1998). "Maternal determinants of seed dispersal in *Cakile edentula*: Fruit, plant, and site traits." Ecology **79**(8): 2771-2788.
- Donohue, K. (2003). "The influence of neighbor relatedness on multilevel selection in the Great Lakes sea rocket." American Naturalist **162**(1): 77-92.
- Dudley, S. A. and A. L. File (2007). "Kin recognition in an annual plant." Biology Letters **3**: 435–438.
- Dudley, S. A. and J. Schmitt (1996). "Testing the Adaptive Plasticity Hypothesis - Density-Dependent Selection on Manipulated Stem Length in *Impatiens capensis*." American Naturalist **147**(3): 445-465.
- Eissenstat, D. M. (1991). "On the relationship between specific root length and the rate of root proliferation: A field study using citrus rootstocks." New Phytologist **118**: 63-68.
- Falik, O., P. Reides, M. Gersani and A. Novoplansky (2003). "Self/non-self discrimination in roots." Journal of Ecology **91**(4): 525-531.
- Fang, S. Q., R. T. Clark, Y. Zheng, A. S. Iyer-Pascuzzi, J. S. Weitz, L. V. Kochian, H. Edelsbrunner, H. Liao and P. N. Benfey (2013). "Genotypic recognition and spatial responses by rice roots." Proceedings of the National Academy of Sciences of the United States of America **110**(7): 2670-2675
- Farley, R. A. and A. H. Fitter (1999). "The responses of seven co-occurring woodland herbaceous perennials to localized nutrient-rich patches." Journal of Ecology **87**(5): 849-859.

- Fitter, A., T. Strickland, M. Harvey and G. Wilson (1991). "Architectural analysis of plant root systems 1. Architectural correlates of exploitation efficiency." New Phytologist **118**: 375-382
- Flores, H. E., J. M. Vivanco and V. M. Loyola-Vargas (1999). "[]Radicle' biochemistry: the biology of root-specific metabolism." Trends in Plant Science **4**(6): 220-226.
- Franklin, K. A. and G. C. Whitelam (2005). "Phytochromes and shade-avoidance responses in plants." Annals of Botany **96**(2): 169-175.
- Gersani, M., Z. Abramsky and O. Falik (1998). "Density-dependent habitat selection in plants." Evolutionary Ecology **12**: 223-234.
- Gersani, M., J. S. Brown, E. E. O'Brien, G. M. Maina and Z. Abramsky (2001). "Tragedy of the commons as a result of root competition." Journal of Ecology **89**: 660-669.
- Grime, J. P., J. C. Crick and J. E. Rincon (1986). The ecological significance of plasticity. Plasticity in plants, D.H. Jennings and A.J. Trewavas (eds.). Scarborough, North Yorkshire, Pindar: 5-29.
- Gruntman, M. and A. Novoplansky (2004). "Physiologically mediated self/non-self discrimination in roots." Proc Nat Acad Sci Usa **101**: 3863-3867.
- Hamilton, W. D. (1964). "The genetical evolution of social behavior, I & II." Journal of Theoretical Biology **7**: 1-52.
- Hodge, A. (2004). "The plastic plant: root responses to heterogeneous supplies of nutrients." New Phytologist **162**(1): 9-24.
- Hodge, A. (2009). "Root decisions." Plant Cell and Environment **32**(6): 628-640.
- Hodge, A., J. Stewart, D. Robinson, B. S. Griffiths and A. H. Fitter (2000). "Competition between roots and soil micro-organisms for nutrients from nitrogen-rich patches of varying complexity." Journal of Ecology **88**(1): 150-164.
- Hodge, A., J. Stewart, D. Robinson, B. S. Griffiths and A. H. Fitter (2000). "Spatial and physical heterogeneity of N supply from soil does not influence N capture by two grass species." Functional Ecology **14**(5): 645-653.

- Holzappel, C. and P. Alpert (2003). "Root cooperation in a clonal plant: connected strawberries segregate roots." Oecologia **134**: 72-77.
- Huber-Sannwald, E., D. A. Pyke and M. M. Caldwell (1996). "Morphological plasticity following species-specific recognition and competition in two perennial grasses." American Journal of Botany **83**: 919-931.
- Jackson, R. B. and M. M. Caldwell (1989). "The timing and degree of root proliferation in fertile soil microsites for three cold-desert perennials." Oecologia **81**: 149-153.
- Keddy, P. A. (1982). "Population ecology on an environmental gradient: *Cakile edentula* on a sand dune." Oecologia **52**: 348-355.
- Kelly, J. K. (1996). "Kin selection in the annual plant *Impatiens capensis*." American Naturalist **147**(6): 899-918.
- Lomnicki, A. (1988). "The Place of Modeling in Ecology." Oikos **52**(2): 139-142.
- Lynch, J. (1995). "Root architecture and plant productivity." Plant Physiology **109**: 7-13.
- Mahall, B. E. and R. M. Callaway (1996). "Effects of regional origin and genotype in intraspecific root communication in the desert shrub *Ambrosia dumosa* (Asteraceae)." American Journal of Botany **83**: 93-98.
- Maina, G. G., J. S. Brown and M. Gersani (2002). "Intra-plant versus inter-plant root competition in beans: avoidance, resource matching or tragedy of the commons." Plant Ecology **160**(2): 235-247.
- Malamy, J. E. (2005). "Intrinsic and environmental response pathways that regulate root system architecture." Plant Cell and Environment **28**(1): 67-77.
- Murphy, G. P. and S. A. Dudley (2007). "Above- and below-ground competition cues elicit independent responses." Journal of Ecology **95**(2): 261-272.
- Murphy, G. P. and S. A. Dudley (2009). "Kin recognition: competition and cooperation in *Impatiens* (Balsaminaceae)." American Journal of Botany **in press**.
- Neufeld, H. S., D. M. Durall, P. M. Rich and D. T. Tingey (1989). "A Rootbox for Quantitative Observations on Intact Entire Root Systems." Plant and Soil **117**(2): 295-298

- Novoplansky, A. (2009). "Picking battles wisely: plant behaviour under competition." Plant Cell and Environment **32**(6): 726-741.
- O'Brien, E. E., M. Gersani and J. S. Brown (2005). "Root proliferation and seed yield in response to spatial heterogeneity of below-ground competition." New Phytologist **168**: 401-412.
- Payne, A. M. and M. A. Maun (1981). "Dispersal and floating ability of dimorphic fruit segments of *Cakile edentula* var *lacustris*." Canadian Journal of Botany **59**: 2595-2602.
- Pfennig, D. W., H. K. Reeve and P. W. Sherman (1993). "Kin Recognition and Cannibalism in Spadefoot Toad Tadpoles." Animal Behaviour **46**(1): 87-94.
- Rodman, J. E. (1974). "Systematics and evolution of the genus *Cakile* (Cruciferae)." Contributions of the Gray Herbarium, Harvard University **205**: 3-146.
- Smith, H. (1982). "Light Quality, Photoperception, and Plant Strategy." Annual Review of Plant Physiology and Plant Molecular Biology **33**: 481-518.
- Trewavas, A. (2009). "What is plant behaviour?" Plant Cell and Environment **32**(6): 606-616.
- Waldman, B. (1988). "The ecology of kin recognition." Annual Review of Ecology and Systematics **19**: 543-571.
- Walker, T. S., H. P. Bais, K. M. Halligan, F. R. Stermitz and J. M. Vivanco (2003). "Metabolic profiling of root exudates of *Arabidopsis thaliana*." Journal of Agricultural and Food Chemistry **51**(9): 2548-2554.
- Weinig, C. and L. F. Delph (2001). "Phenotypic plasticity early in life constrains developmental responses later." Evolution **55**(5): 930-936.
- West, S. A. and A. Gardner (2010). "Altruism, Spite, and Greenbeards." Science **327**(5971): 1341-1344.
- Whitelam, G. C. and H. Smith (1991). "Retention of phytochrome-mediated shade avoidance responses in phytochrome-deficient mutants of *Arabidopsis*, cucumber and tomato." Journal of Plant Physiology **139**: 119-125.